SHOUT
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BIOLOGICAL BULLETIN

OF THE

Marine Biological Laboratory

WOODS HOLE, MASS.

Editorial Staff

E. G. Conklin—*Princeton University.*
George T. Moore—*The Missouri Botanic Garden.*
T. H. Morgan—*Columbia University.*
W. M. Wheeler—*Harvard University.*
E. B. Wilson—*Columbia University.*

Managing Editor

Frank R. Lillie—*The University of Chicago.*

Volume XXXIX.

WOODS HOLE, MASS.
JULY TO DECEMBER, 1920

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BIOLOGICAL BULLETIN

THE MARINE BIOLOGICAL LABORATORY

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I. TRUSTEES

EX OFFICIO

FRANK R. LILLIE, *Director*, The University of Chicago.
GILMAN A. DREW, *Assistant Director*, Marine Biological Laboratory.
D. BLAKELY HOAR, *Treasurer*, 161 Devonshire Street, Boston, Mass.
GARY N. CALKINS, *Clerk of the Corporation*, Columbia University.

TO SERVE UNTIL 1923

H. C. BUMPUS, Brown University.
R. A. HARPER, Columbia University.
W. A. LOCY, Northwestern University.
JACQUES LOEB, The Rockefeller Institute for Medical Research.
GEORGE T. MOORE, Missouri Botanical Garden, St. Louis.
L. L. NUNN, Telluride, Colo.
W. J. V. OSTERHOUT, Harvard University.
W. M. WHEELER, Bussey Institution, Harvard University.

TO SERVE UNTIL 1922

CORNELIA M. CLAPP, Mount Holyoke College.
E. G. CONKLIN, Princeton University.

Ross G. Harrison, Yale University.
Camillus G. Kidder, 27 William Street, New York City.
M. M. Metcalf, Oberlin, Ohio.
William Patten, Dartmouth College.
Jacob Reighard, University of Michigan.
W. B. Scott, Princeton University.

TO SERVE UNTIL 1921

S. F. Clarke, Williamstown, Mass.
Charles A. Coolidge, Ames Building, Boston, Mass.
C. R. Crane, Woods Hole, Mass., *President of the Corporation.*
Alfred G. Mayor, Carnegie Institution.
C. E. McClung, University of Pennsylvania.
T. H. Morgan, Columbia University.
Erwin F. Smith, United States Department of Agriculture.
E. B. Wilson, Columbia University.

TO SERVE UNTIL 1920

H. H. Donaldson, Wistar Institute of Anatomy and Biology.
M. J. Greenman, Wistar Institute of Anatomy and Biology.
C. W. Hargitt, Syracuse University.
H. S. Jennings, Johns Hopkins University.
George Lefevre, University of Missouri, *Secretary of the Board.*
A. P. Mathews, The University of Chicago.
G. H. Parker, Harvard University.
Henry B. Ward, University of Illinois.

II. ACT OF INCORPORATION

No. 3170.

Commonwealth of Massachusetts

Be It Known, That whereas Alpheus Hyatt, William Sanford Stevens, William T. Sedgwick, Edward G. Gardiner, Susan Minns, Charles Sedgwick Minot, Samuel Wells, William G. Farlow, Anna D. Phillips and B. H. Van Vleck have associated themselves with the intention of forming a Corporation under the name of the Marine Biological Laboratory, for the purpose of establishing and maintaining a laboratory or station for scientific study and investigation, and a school for instruction in biology and natural history, and have complied with the provisions of the statutes of this Commonwealth in such case made and provided, as appears from the certificate of the President, Treasurer, and Trustees of said Corporation, duly approved by the Commissioner of Corporations, and recorded in this office;

Now, therefore, I, HENRY B. PIERCE, Secretary of the Commonwealth of Massachusetts, *do hereby certify* that said A. Hyatt, W. S. Stevens, W. T. Sedgwick, E. G. Gardiner, S. Minns, C. S. Minot, S. Wells, W. G. Farlow, A. D. Phillips, and B. H. Van Vleck, their associates and successors, are legally organized and established as, and are hereby made, an existing Corporation, under the name of the MARINE BIOLOGICAL LABORATORY, with the powers, rights, and privileges, and subject to the limitations, duties, and restrictions, which by law appertain thereto.

Witness my official signature hereunto subscribed, and the seal of the Commonwealth of Massachusetts hereunto affixed, this twentieth day of March, in the year of our LORD ONE THOUSAND, EIGHT HUNDRED and EIGHTY-EIGHT. HENRY B. PIERCE,

[SEAL.] *Secretary of the Commonwealth.*

III. BY-LAWS OF THE CORPORATION OF THE MARINE BIOLOGICAL LABORATORY

I. The annual meeting of the members shall be held on the second Tuesday in August, at the Laboratory, in Woods Hole, Mass., at 12 o'clock noon, in each year, and at such meeting the members shall choose by ballot a Treasurer and a Clerk, who shall be, *ex officio*, members of the Board of Trustees, and Trustees as hereinafter provided. At the annual meeting to be held in 1897, not more than twenty-four Trustees shall be chosen, who shall be divided into four classes, to serve one, two, three, and four years, respectively, and thereafter not more than eight Trustees shall be chosen annually for the term of four years. These officers shall hold their respective offices until others are chosen and qualified in their stead. The Director and Assistant Director, who shall be chosen by the Trustees, shall also be Trustees, *ex officio*.

II. Special meetings of the members may be called by the Trustees, to be held in Boston or in Woods Hole at such time and place as may be designated.

III. The Clerk shall give notice of meetings of the members by publication in some daily newspaper published in Boston at least fifteen days before such meeting, and in case of a special meeting the notice shall state the purpose for which it is called.

IV. Twenty-five members shall constitute a quorum at any meeting.

V. The Trustees shall have the control and management of the affairs of the Corporation; they shall present a report of its condition at every annual meeting; they shall elect one of their number President and may choose such other officers and agents as they may think best; they may fix the compensation and define the duties of all the officers and agents; and may remove them, or any of them, except those chosen by the members, at any time; they may fill vacancies occurring in any manner in their own number or in any of the offices. They shall from time to time elect members to the Corporation upon such terms and conditions as they may think best.

VI. Meetings of the Trustees shall be called by the President, or by any two Trustees, and the Secretary shall give notice thereof by written or printed notice sent to each Trustee by mail, postpaid. Seven Trustees shall constitute a quorum for the transaction of business. The Board of Trustees shall have power to choose an Executive Committee from their own number, and to delegate to such Committee such of their own powers as they may deem expedient.

VII. The President shall annually appoint two Trustees, who shall constitute a committee on finance, to examine from time to time the books and accounts of the Treasurer, and to audit his accounts at the close of the year. No investments of the funds of the Corporation shall be made by the Treasurer except approved by the finance committee in writing.

VIII. The consent of every Trustee shall be necessary to dissolution of the Marine Biological Laboratory. In case of dissolution, the property shall be given to the Boston Society of Natural History, or some similar public institution, on such terms as may then be agreed upon.

IX. These By-Laws may be altered at any meeting of the Trustees, provided that the notice of such meeting shall state that an alteration of the By-Laws will be acted upon.

X. Any member in good standing may vote at any meeting, either in person or by proxy duly executed.

IV. THE TREASURER'S REPORT

MARINE BIOLOGICAL LABORATORY BALANCE SHEET, DECEMBER 31, 1919

Assets

Cash in bank	$ 14,077.69	
Petty cash at Woods Hole	200.00	$ 14,277.69
Notes receivable (secured)		1,000.00
Accounts receivable		10,178.23
Inventories (not available)		
Items in suspense		131.82
Investments:		
Securities (Schedule I.)	11,768.83	
Cash (Schedule II.)	814.58	12,583.41
Stock in General Biological Supply Company, Inc.		10,000.00
Gansett property account	22,559.88	
Less mortgage	14,629.21	7,930.67
Educational plant (Schedule III.):		
Land	95,856.14	
Buildings	174,713.44	
Equipment	78,179.37	
	348,748.95	
Less reserve for depreciation	28,543.46	320,205.49
		$376,307.31

Liabilities

Accounts payable accrued (estimated)		$ 1,300.00
Notes payable issued in part payment of shares of General Biological Supply Company, Inc.	$ 4,500.00	
Less amounts paid thereon	2,000.00	2,500.00
Notes payable issued on account of purchase of investment securities	2,000.00	
Trust funds	10,583.41	12,583.41
Balancing account:		
Balance, January 1	347,558.18	
Special donations	7,500.00	
Excess of income for year (Exhibit B)	4,314.79	
New equipment transferred from expense	550.93	359,923.90
		$376,307.31

MARINE BIOLOGICAL LABORARORY, INCOME AND EXPENSE FOR YEAR ENDED DECEMBER 31, 1919

		Expense	Income	Loss	Gain
Administrative expense		$ 6,556.84		$ 6,556.84	
Bar Neck property expense		168.00		168.00	
BIOLOGICAL BULLETIN and annual dues		2,773.38	2,972.34		198.96
Carpenter department		858.14	35.05	823.09	
Chemical department		1,422.80		1,422.80	
Dexter House special expense		141.95		141.95	
Dormitory department		1,874.87	2,083.93		209.06
Instruction		5,023.36	6,395.00		1,371.64
Interest on notes payable		142.54		142.54	
Lectures, evening		90.65		90.65	
Lectures, philosophical		100.00		100.00	
Library department		2,185.50		2,185.50	
Maintenance, buildings and grounds		4,997.99		4,997.99	
Mess		21,302.46	21,462.32		159.86
New laboratory expense		3,760.02		3,760.02	
Newman cottage		50.41		50.41	
Pumping station expense		374.01		374.01	
Research department		2,045.15	4,250.00		2,204.85
Sundry expense and income		1,611.13	6,437.85		4,826.72
Supply department:					
General expense	$20,438.36				
Administration department expense	1,200.00				
Boats	6,064.04				
Fish trap	1,365.78				
Maintenance, buildings and grounds	200.00				
Pumping station	187.00				
Truck	507.23				
Rental and depreciation	2,503.80	32,466.21	36,671.08		4,204.87
Truck		507.23		507.23	
Total current expenses		88,452.64	80,307.57	21,321.03	13,175.96
Total income		80,307.57		13,175.96	
Excess of expenses		$ 8,145.07		8,145.07	
Depreciation		7,403.22			
Accounts charged off		396.92			
		15,945.21			
Contribution by Friendship Fund, Inc.	$20,000.00				
Others	260.00	20,260.00			
Balance to balancing account		4,314.79			

MARINE BIOLOGICAL LABORATORY INVESTMENTS (BOOK VALUES), DECEMBER 31, 1919

Reserve Fund

$3,000 American Telephone & Telegraph Company, 4's (represented by receipts for same as collateral)	$2,921.25	
500 Western Telephone & Telegraph Company, 5's	496.88	
6 shares American Smelting and Refining Company, Preferred (receipt on file)	732.00	
8 shares General Electric Company	948.05	
14 shares United Shoe Machinery Company, Preferred	393.75	
5 shares Massachusetts Gas Companies, Preferred	444.63	$ 5,936.56

Lucretia Crocker Fund

$300 Liberty Loan Bonds, 4¼'s	300.00	
1/5 of $1,000 American Telephone & Telegraph Company, 4's	194.75	
18 shares Vermont & Massachusetts Railway Company	2,416.50	
1 share American Telephone & Telegraph Company	125.02	
3 shares General Electric Company	364.85	
1 share West End Street Railway Company	83.00	3,484.12

Library Fund

$300 Liberty Loan Bonds, 4¼'s	300.00	
4/5 of $1,000 American Telephone & Telegraph Company, 4's	779.00	
3 shares American Telephone & Telegraph Company	375.04	
1 share American Smelting and Refining Company, Preferred (receipt on file)	122.00	
3 shares General Electric Company	362.10	
5 shares United Shoe Machinery Company, Preferred	140.63	
3 shares Massachusetts Gas Companies, Preferred	269.38	2,348.15
Total, Exhibit A		$11,768.83

January 22, 1920

MR. D. BLAKELY HOAR,
161 Devonshire Street,
Boston.

Dear Sir: We have completed our audit of the accounts of the Marine Biological Laboratory for the year ended December 31, 1919, as kept both at your office in Boston and at Woods Hole, and report thereon in the accompanying exhibits and schedules:

Exhibit A—Balance-Sheet as of December 31, 1919.
B—Income-and-Expense for the Year ended December 31, 1919.

Schedule I—Investments (Book Values).
II—Cash Receipts and Payments on Account of Investments.
III—Summary of Inventory of Land, Buildings, and Equipment.
IV—Depreciation Reserve.

We certify that, subject to the comments herewith, the balance-sheet and income-and-expense statement shown in Exhibits A and B are in accordance with the books and correct to the best of our knowledge and belief.

Very respectfully,
(*Signed*) Harvey S. Chase & Company,
Certified Public Accountants.

V. THE DIRECTOR'S REPORT

January 1, 1920

To the Trustees of the Marine Biological Laboratory:

Gentlemen: I beg to present herewith a report of the thirty-second session of the Laboratory for the year 1919: The conclusion of the war found the Laboratory with its organization intact and ready to continue its work. It was anticipated that there would probably be a rather slow recovery of ground lost during the war, and we were hardly prepared for an influx of students and investigators that carried the attendance beyond the previous high water mark. The demand for working places by investigators and students was, however, so large that it was necessary to refuse a considerable number of applications. The Laboratory has in fact outgrown its accommodations in all departments.

It is felt by most members of the Laboratory that the student attendance should not be increased, and that the tendency to overcrowding must be dealt with by yet more rigorous requirements. On the research side, the situation is more serious; it is practically impossible to accommodate all well qualified investigators who apply, and the effort to do so results in undue crowding. This situation is aggravated by the fact that the old wooden buildings, to which the great majority of the investigators must be assigned, are increasingly ill-adapted to the changing requirements of biological research. Another large, firmly constructed, fireproof building has become a necessity.

At the time of the organization of the Division of Biology and Agriculture of the National Research Council, the needs of the Marine Biological Laboratory for additional support were presented by the Director, and a committee of the Division was appointed which investigated the situation and recommended that the National Research Council lend its aid in securing funds for the erection of a new building and for extension of the library. The report was considered by the Executive Committee of the Division and passed on with its approval and endorsement to the Executive board of the Council which functions for the whole organization. The report was then thoroughly considered and heartily approved by the Executive Board.

The support of the National Research Council is thus definitely pledged to our projects for a new building and extension of the library, but this does not relieve us of the necessity of using our own efforts for the attainment of these immediate objects. The National Research Council does not possess funds of its own for such projects, but uses its great influence to reinforce the best projects of scientific research. With such endorsement our efforts should be rewarded more rapidly than we could otherwise expect.

The tabular view of attendance (p. 20) shows a total attendance of 262 (students 128, and investigators 134) which is twenty greater than the previous largest year (1915). This number cannot be exceeded until another laboratory is built. The receipts from students and investigators were $10,545 in 1919 as compared with $8,725 in 1916, $7,850 in 1917, and $6,530 in 1918. The number of subscribing institutions was 47 in 1919; the largest previous number was 40 in 1916; in 1918 the number was 33.

During the year the business of the Supply Department was reorganized in such a way as to charge to it all items, hitherto carried on the books as general expense items, with which it was properly accountable; and as an offset it was credited with supplies furnished students and investigators, which had previously not been accounted for. It was thought thus to place the department on a sound business basis, so that in its future development no uneasiness might be felt by its expansion. It was found necessary also during the year to raise the general price level 40 per cent., by two successive 20 per cent. increments, over pre-war figures. The items charged to the Department are the following: General expense (including salaries and supplies), administration expense, boat expense, fish trap expense, maintenance of buildings and grounds of the department, proportional share of pumping expense, truck, rental and depreciation. The credits of the Supply Department consist of sales and (for 1919) a credit of $3,000, the estimated value of material furnished students and investigators. The total income of the Supply Department on this basis for 1919 amounted to $36,671.08, and the total expense to $32,466.21. The previous largest year's business of the Department was $21,096.65 in 1916. Making due allowance for the $3,000 credit in 1919, not previously allowed, and also for the 40 per cent. increase in prices, operative only during the latter half of the year, it will be seen that a most gratifying increase in business was effected.

The development of this department requires additional laboratory space and better winter quarters for the personnel. Therefore with the approval of the Board of Trustees an additional frame building adjacent to the present quarters was begun in the fall of 1919. This building will provide for all the office and preparation work of the Department, and the old building will be used mainly for storage.

Another step in the extension of the Supply Department during the year was purchase of a controlling interest in the General Biological Supply House located in Chicago. This house was organized by Dr. M. M. Wells, formerly Assistant Professor of Zoölogy in the University of Chicago, and is managed entirely by University trained men who can be trusted to maintain high standards. It was hoped to accomplish several purposes by this venture; first, by making Chicago a distributing center for the West considerable savings in time of delivery and in shipping expense can be accomplished; in the second place, new lines of supplies such as microscopic slides and lantern slides can be developed without enlarging the Woods Hole organization; the market for Woods Hole supplies should also be largely increased by the better attention to marketing that can be given by a strictly business organization.

During the year Dr. H. McE. Knower, who had acted for many years as librarian offered his resignation. During his term of office the library has undergone its greatest development, and owing to his efforts it has been well arranged and catalogued. The Board of Trustees in accepting Dr. Knower's resignation instructed the Secretary to express to him the appreciation which all felt of the value of his services to the Laboratory. The office of librarian was filled by the appointment of Dr. R. P. Bigelow, librarian of the Massachusetts Institute of Technology. Mrs. T. H. Montgomery, Jr. was appointed resident assistant librarian in immediate charge of the library. At the meeting of the Corporation August 12, 1919, Professor W. M. Wheeler, of the Bussey Institution, Harvard University, was elected a member of the Board of Trustees of the class of 1923 to succeed Dr. John C. Phillips

Attention is called to the very satisfactory report of the Treasurer (p. 5), and to the list of the Staff, of Investigators and Students, the Tabular View of Attendance, the Subscribing Institutions, the evening lectures, and of the Members of the Corporation which are appended as parts of the Director's report.

1. THE STAFF

1919

FRANK R. LILLIE, Director, Professor of Embryology, and Chairman of the Department of Zoölogy, The University of Chicago.

GILMAN A. DREW, Assistant Director, Marine Biological Laboratory.

ZOÖLOGY

I. INVESTIGATION

GARY N. CALKINS, Professor of Protozoölogy, Columbia University.

E. G. CONKLIN, Professor of Zoölogy, Princeton University.

GILMAN A. DREW, Assistant Director, Marine Biological Laboratory.

George Lefevre, Professor of Zoölogy, The University of Missouri.

Frank R. Lillie, Professor of Embryology, The University of Chicago.

C. E. McClung, Professor of Zoölogy, University of Pennsylvania.

T. H. Morgan, Professor of Experimental Zoölogy, Columbia University.

E. B. Wilson, Professor of Zoölogy, Columbia University.

II. INSTRUCTION

W. C. Allee, Professor of Biology, Lake Forest College.

George A. Baitsell, Instructor in Biology, Yale University.

Robert H. Bowen, Graduate Student, Columbia University.

Robert Chambers, Jr., Cornell University Medical College.

Ann H. Morgan, Professor of Zoölogy, Mount Holyoke College.

Charles L. Parmenter, Research Fellow, University of Pennsylvania.

W. J. Crozier, Assistant Professor of Zoölogy, The University of Chicago.

PROTOZOÖLOGY

Gary N. Calkins, Professor of Protozoölogy, Columbia University.

Louise H. Gregory, Assistant Professor of Zoölogy, Barnard College.

EMBRYOLOGY

I. INVESTIGATION

(*See Zoölogy*)

II. INSTRUCTION

Gilman A. Drew, Assistant Director, Marine Biological Laboratory.

Charles G. Rogers, Professor of Comparative Physiology, Oberlin College.

Hubert B. Goodrich, Associate Professor of Zoölogy, Wesleyan University.

Elizabeth A. Smith, Assistant Professor, University of Wisconsin.

B. H. Grave, Professor of Biology, Knox College.

PHYSIOLOGY

I. INVESTIGATION

Albert P. Mathews, Professor of Biochemistry, The University of Cincinnati.

Ralph S. Lillie, Professor of Biology, Clark University.

Harold C. Bradley, Professor of Physiological Chemistry, University of Wisconsin.

II. INSTRUCTION

Ralph S. Lillie, Professor of Biology, Clark University.

Frank P. Knowlton, Professor of Physiology, Syracuse University.

A. R. Moore, Professor of Physiology, Rutgers College.

PHILOSOPHICAL ASPECTS OF BIOLOGY AND ALLIED SCIENCES

LECTURES

Edward G. Spaulding, Professor of Philosophy, Princeton University.

BOTANY

I. INVESTIGATION

Edward M. East, Professor of Experimental Plant Morphology, Harvard University.

Robert A. Harper, Professor of Botany, Columbia University.

E. Newton Harvey, Professor of Physiology, Princeton University.

Winthrop J. V. Osterhout, Professor of Botany, Harvard University.

II. INSTRUCTION

Ivey F. Lewis, Professor of Biology, University of Virginia.

William D. Hoyt, Associate Professor of Biology, Washington and Lee University.

Wm. Randolph Taylor, Instructor in Botany, University of Pennsylvania.

LIBRARY

H. McE. Knower, Professor of Anatomy, University of Cincinnati.

Priscilla B. Montgomery, Assistant Librarian.

CHEMICAL SUPPLIES

Oliver S. Strong, Assistant Professor of Neurology, College of Physicians and Surgeons, New York City, Chemist.

SUPPLY DEPARTMENT

G. M. Gray, Curator.

John J. Veeder, Captain.

E. M. Lewis, Engineer.

A. W. Leathers, Collector.

A. M. Hilton, Collector.

J. McInnis, Collector.

F. G. Gustafson, Collector in Botany.

Edna E. Wells, Clerk.

F. M. MacNaught, Business Manager.

Herbert A. Hilton, Superintendent of Buildings and Grounds.

2. STUDENTS AND INVESTIGATORS—1919

INDEPENDENT INVESTIGATORS—ZOÖLOGY

Ackert, James E., Professor of Zoölogy, Kansas State Agricultural College.

Addison, William H. F., Professor of Normal Histology and Embryology, University of Pennsylvania.

Allee, Warder C., Professor of Biology, Lake Forest College.

Allen, Ezra, University of Pennsylvania.

Altenburg, Edgar, Instructor in Biology, Rice Institute.

Baitsell, George A., Assistant Professor of Biology, Yale University.

Beckwith, Cora J., Associate Professor of Zoölogy, Vassar College.

Bridges, Calvin B., Research Assistant, Columbia University.

Calkins, Gary N., Professor of Protozoölogy, Columbia University.

Chambers, Robert, Assistant Professor of Anatomy, Cornell University Medical College.

Charlton, Harry H., Acting Director of Anatomical Dept., Fordham University.

Clapp, Cornelia M., Emeritus Professor of Zoölogy, Mount Holyoke College.

Clark, Eleanor L., Columbia, Mo.

Clark, Eliot R., Professor of Anatomy, University of Missouri.

Conklin, Edwin G., Professor of Biology, Princeton University.

Copeland, Manton, Professor of Biology, Bowdoin College.

Crozier, William J., Assistant Professor of Zoölogy, University of Chicago.

Curtis, Maynie R., Associate in Cancer Research, Columbia University.

Curtis, W. C., Professor of Zoölogy, University of Missouri.

Danchakoff, Vera, Assistant Professor of Anatomy, College of Physicians and Surgeons.

Dawson, James A., Yale University.

Dodds, Gideon S., Assistant Professor of Histology, West Virginia University.

Donaldson, Henry H., Professor of Neurology, Wistar Institute.

Donaldson, John C., Instructor in Anatomy, University of Cincinnati Medical School.

Drew, Gilman A., Assistant Director, Marine Biological Laboratory, Woods Hole.

Glaser, Otto C., Professor of Biology, Amherst College.

Glaser, Rudolf W., Bussey Institution.

Goodrich, Hubert B., Associate Professor of Zoölogy, Wesleyan University.

Grave, Benjamin H., Professor of Biology, Knox College.

Grave, Caswell, Professor of Zoölogy, Washington University.

Gregory, Louise H., Assistant Professor of Zoölogy, Barnard College.

Heilbrunn, Lewis V., Brooklyn, N. Y.

Holt, Caroline M., Assistant Professor of Biology, Simmons College.

Hyman, Libbie H., Research Assistant, University of Chicago.

Kindred, James E., Instructor in Biology, Western Reserve University.

Knower, Henry McE., Professor of Anatomy, University of Cincinnati.

Lefevre, George, Professor of Zoölogy, University of Missouri.

Lewis, Warren H., Research Associate, Carnegie Institution of Washington.

Lewis, Mrs. Warren H., Baltimore, Md.

Lillie, Frank R., Chairman, Department of Zoölogy, University of Chicago.

Loeb, Leo, Professor of Comparative Pathology, Washington University.

Malone, Edward F., Professor of Histology, University of Cincinnati.
Mast, Samuel O., Professor of Zoölogy, Johns Hopkins University.
Mavor, James W., Professor of Zoölogy, Union College.
McClung, Clarence E., Chairman Division of Biology.and Agriculture, National Research Council.
Metcalf, Maynard M., Oberlin College.
Metz, Charles W., Research Associate, Carnegie Institution, Cold Spring Harbor.
Morgan, Ann H., Professor of Zoölogy, Mount Holyoke College.
Morgan, Thomas H., Professor of Experimental Zoölogy, Columbia University.
Morrill, Charles V., Instructor in Anatomy, Cornell Medical College.
Muller, Hermann J., Instructor, Columbia University.
Nabours, Robert K., Professor of Zoölogy, Kansas Agricultural College.
Parmenter, Charles L., Instructor, University of Pennsylvania.
Patten, William, Professor of Biology, Dartmouth College.
Pinney, Mary E., Professor of Biology, Lake Erie College.
Plough, Harold H., Associate Professor of Biology, Amherst College.
Richards, A., Professor of Zoölogy, Wabash College.
Rogers, Charles G., Professor of Comparative Physiology, Oberlin College.
Schrader, Franz, Columbia University.
Smith, Elizabeth A., Assistant Professor, University of Wisconsin.
Spaulding, Edward G., Professor of Philosophy, Princeton University.
Speidel, Carl C., Professor, St. Lawrence University.
Stockard, Charles R., Professor of Anatomy, Cornell University Medical College.
Sturtevant, Alfred H., Research Assistant, Columbia University.
Weinstein, Alexander, Investigator, Carnegie Institution.
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Whiting, Phineas W., Professor of Biology, Franklin and Marshall College.
Wieman, Harry L., Professor of Zoölogy, University of Cincinnati.
Wilson, Edward B., Professor of Zoölogy, Columbia University.

BEGINNING INVESTIGATORS—ZOÖLOGY

Adams, A. Elizabeth, Associate Professor of Zoölogy, Mount Holyoke College.
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Daniels, Helen, Assistant, Columbia University.
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Lancefield, Donald E., Assistant in Zoölogy, Carnegie Institution.
Mason, Eleanor D., Assistant, Carnegie Institution, Cold Spring Harbor.
Matsui, Kenkichi, Imperial College of Agriculture, Japan.
Nonidez, Jose F., Research Associate, Carnegie Institution.
Patten, Mary W., Assistant, Yale University.
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Reed, Phoebe C., Assistant, Carnegie Institution.
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Safir, Shelley R., Teacher, Stuyvesant High School, New York City.
Shaffer, Elmer L., Procter Fellow in Biology, Princeton University.
Wallace, Edith M., Research Assistant, Columbia University.
Willier, Benjamin H., University of Chicago.

INDEPENDENT INVESTIGATORS—PHYSIOLOGY

Baldwin, Francis M., Assistant Professor, Iowa State College.
Bradley, Harold C., Professor of Physiological Chemistry, University of Wisconsin.
Clowes, G. H. A., Director and Consulting Chemist, Research Laboratory, Eli Lilly & Co.
Edwards, Dayton J., Lecturer in Physiology, Cornell University Medical College.
Ellis, Frederick W., Monson, Mass.
Forbes, Alexander, Instructor in Physiology, Harvard Medical School.
Garrey, Walter E., Professor of Physiology, Tulane University.
Harvey, E. Newton, Professor of Physiology, Princeton University.
Hecht, Selig, Assistant Professor, Creighton University.
Hyde, Ida H., Professor of Physiology, University of Kansas.
Irwin, Marian, Radcliffe College.
Just, E. E., Professor of Physiology, Howard University.
Knowlton, F. P., Professor of Physiology, Syracuse University.
Lillie, Ralph S., Professor of Biology, Clark University.
Loeb, Jacques, Head of Division of Experimental Biology, Rockefeller Institute.
Lyon, Elias P., Professor of Physiology, University of Minnesota.
Mathews, Albert P., Professor of Biochemistry, University of Cincinnati.
Maxwell, S. S., Associate Professor of Physiology, University of California
Moore, A. R., Professor of Physiology, Rutgers College.
Pratt, Frederick H., Honorary Fellow in Biology, Clark University.
Redfield, Alfred C., Assistant Professor, University of Toronto.
Spaeth, Reynold A., Associate in Physiology, Johns Hopkins University.
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BEGINNING INVESTIGATORS—PHYSIOLOGY

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Pond, Samuel E., Fellow, Clark University.
Sampson, Myra M., Assistant Professor, Smith College.
Starr, Isaac, Jr., University of Pennsylvania.
Stone, Amy E., Radcliffe College.

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Brooks, Sumner C., Research Fellow, Harvard Medical School.
Fenn, Wallace O., Harvard Medical School.

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Hazen, Tracy E., Assistant Professor of Botany, Columbia University.
Hoyt, William D., Associate Professor of Biology, Washington and Lee Univ.
Lewis, Ivey F., Professor of Biology, University of Virginia.
Osterhout, Winthrop J. V., Professor of Botany, Harvard University.

BEGINNING INVESTIGATORS—BOTANY

Brooks, Mrs. S. C., Boston, Mass.
Gluck, Marguerite L., Columbia University.
Holland, Dorothy F., Summit, N. J.
Taylor, William R., Instructor in Botany, University of Pennsylvania.

1919

STUDENTS

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Allen, Agnes L., Student, Mount Holyoke College.
Avins, Emma R., Student, Oberlin College.
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Bergner, Dorothy, Student, Goucher College.
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Ellis, Mildred, Student, Radcliffe College.
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Hoadley, Leigh, Student, University of Michigan.
Hoffman, Agnes, Lake Forest College.
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Sheldon, Margaret, Student, Oberlin College.
Smith, Catharine, Smith College.
Springer, Mary G., Student, Oberlin College.
St. Clair, Harriet W., Student, Vassar College.
Stacey, Helen R., Simmons College.
Sternberg, Lillian, Student, Barnard College.
Summerill, Verna B., Student, Goucher College.
Townsend, Eric B., Student, Swarthmore College.
Tyler, Jocelyn, Student, Oberlin College.
Watt, Lucy J., Western College for Women.
Watts, Margaret V., Student, Hunter College.
White, Clarissa D., Barnard College.
Wilder, Isabelle M., Student, Wheaton College.
Wondergem, Henry E., Student, University of Rochester.
Woodbridge, Helen, Student, Mount Holyoke College.
Zoeller, Adelaide M., Student. Sophie Newcomb College.

PROTOZOÖLOGY

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Bell, Mary M., Instructor, Wellesley College.
Cutright, Frank, West Virginia Wesleyan College.
Finley, Charles W., Lincoln School of Teachers College, New York.
Fisher, Mary J., Instructor in Zoölogy, Cornell University.
Montanus, John J., Columbia University.
Nute, Bertha E., Torrington, Conn.
Rhodes, Robert C., Professor of Biology, Emory University.
Shields, Walter J., Columbia University.
Shumway, Waldo, Assistant Professor of Biology, Dartmouth College.
Stockman, Charles C., 2d, Instructor in Public Health, Yale University.
Stunkard, H. W., Assistant Professor, University of Illinois.
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Brown, Leland A., Student, Denison University.

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Conklin, Mary, Goucher College.
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Ebeling, Karl W., Johns Hopkins University.
Elliott, Margaret, Knox College.
Folger, Harry T., Assistant, Indiana University.
Freas, Ella M., Mount Holyoke College.
Gates, Gordon E., Foxcroft, Maine.
Gilson, Arthur S., Jr., Dartmouth College.
Greene, Lois D., Student, Grinnell College.
Heizer, Helen S., Student, Radcliffe College.
Hunter, Isabelle L., Student, Simmons College.
Kimball, Mary A., Student Simmons College.
Kreidel, George A., Instructor, St. Joseph's Seminary.
Lang, Dorothy M., University of Vermont.
Lagemann, Anna E., Student, Barnard College.
Morris, Ruth M., Wellesley College.
Roehm, Hilda E., Wellesley College.
Stehle, Mabel E., Instructor in Zoölogy, Clemson College.
Stevens, Kenneth P., Wesleyan University.
Stewart, Walter B., Student, Princeton University.
Swett, Francis H., Laboratory Assistant, Yale University.
Tuers, Russell V., Instructor in Biology, New York University.
Uhlemeyer, Bertha, Assistant in Zoölogy, Washington University.
Waterman, Harriet C., Assistant, Vassar College.
Wheatley, Marjorie A., Laboratory Assistant, New York State Dept. of Health.
White, Gertrude M., Instructor, Carnegie Institute of Technology.

PHYSIOLOGY

Brunswick, David, Harvard Medical School.
Clinton, Isabella M., Woman's Medical College of Pennsylvania.
Dzushi, Hisatake, Student, Amherst College.
Eisenberger, John P., Instructor in Physiology, University of Buffalo.
Gutmann, James, Columbia University.
Gutman, Margaret B., Smith College.
Harrison, Bruce M., Assistant Professor of Zoölogy, Iowa State College.
Hosmer, Helen R., Cleveland, Ohio.
Howard, Frederick H., Professor of Physiology, Williams College.
Jamison, George W., Student, Franklin and Marshall College.
MacCarthy, Kathleen S., Teacher, Hunter College.
Morton, Margaret V., Student, Bryn Mawr College.
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Silverberg, William V., Columbia University.
Spero, Sterling D., Columbia University.
Strang, James M., Massachusetts Institute of Technology.
Sykes, George F., Professor, Oregon Agricultural College.

BOTANY

Dowell, Ruth I., Student, Smith College.
Esty, Frances F., Student, Vassar College.

Graham, Margaret A., Instructor, Hunter College.
Kaan, Helen N., Instructor, Wheaton College.
Klemm, Ralph A., Student, Bucknell University.
Quinnan, Bertha C., Teacher, Pauline Shaw School, Boston.
Webster, Janet, Student, Radcliffe College.
Zirkle, Conway, Student, University of Virginia.

3. TABULAR VIEW OF ATTENDANCE

	1915	1916	1917	1918	1919
Investigators—Total	137	129	129	93	138
Independent:					
Zoölogy	69	70	63	51	69
Physiology	20	23	23	16	24
Botany	6	7	8	5	7
Under Instruction:					
Zoölogy	36	25	24	16	22
Physiology	4	3	6	3	12
Botany	2	1	5	2	4
Students—Total	105	102	83	69	128
Zoölogy	47	50	46	41	55
Protozoölogy	—	—	—	—	15
Embryology	37	26	16	12	33
Physiology	15	14	13	10	17
Botany	6	12	8	6	8
Total Attendance	242	231	212	162	266
Institutions Represented—					
Total	79	73	77	72	88
By investigators	59	51	60	49	61
By students	42	45	36	38	62
Schools and Academies Represented.					
By investigators	3	—	2	—	—
By students	9	3	5	—	4

4. SUBSCRIBING INSTITUTIONS—1919

Amherst College
Barnard College
Bowdoin College
Bryn Mawr College
Butler College

Carnegie Institution of Washington
College of Physicians and Surgeons
Columbia University
Cornell University Medical College
Creighton University
Dartmouth College
Goucher College
Harvard University
Harvard University Medical School
Hunter College (Else Seringhaus Scholarship)
Indiana University
Johns Hopkins University
Kansas State Agricultural College
Lake Forest College
Eli Lilly & Co.
Mount Holyoke College
New York University
Oberlin College
Princeton University
Radcliffe College
Rice Institute
Rockefeller Institute for Medical Research
Simmons College
Smith College
Sophie Newcomb College
University of Chicago
University of Cincinnati
University of Illinois
University of Kansas
University of Michigan
University of Missouri
University of Pennsylvania
University of Rochester
University of Vermont
University of Wisconsin
Vassar College
Wesleyan University
Western Reserve University
Wheaton College
Wistar Institute for Anatomy and Biology
Wellesley College
Yale University

SUBSCRIPTION TABLES

LUCRETIA CROCKER SCHOLARSHIP
FRIEND OF THE LABORATORY

5. EVENING LECTURES, 1919

Tuesday, July 8,
DR. ROBERT CHAMBERS....... "Physical Properties of Protoplasm as Demonstrated by Microdissection."

Friday, July 11,
DR. R. A. EMERSON.......... "The Contribution of Genetics to Studies of Endosperm Development."

Tuesday, July 15,
DR. E. N. HARVEY........... "Bioluminescence" with Demonstrations.

Friday, July 18,
DR. G. H. A. CLOWES......... "Mustard and Other War Gases; their Penetration, Mode of Action and Counteraction in the Body."

Tuesday, July 22,
DR. W. T. BOVIE............. "The Action of Rays on Protoplasm."

Thursday, July 24,
DR. E. S. MORSE............ "The Experiences of a Collector."

Friday, July 25,
DR. C. F. W. MCCLURE....... "The Analysis of Experimental Edema in the Anura."

Tuesday, July 29,
DR. FRANK M. CHAPMAN...... "The Distribution of Bird Life in the Andes."

Friday, Aug. 1,
DR. L. J. HENDERSON......... "The Equilibrium between Oxygen and Carbonic Acid in Blood."

Tuesday, Aug. 5,
DR. C. E. MCCLUNG.......... "Co-operation in Science, and the National Research Council."

Thursday, Aug. 7,
MR. W. LYMAN UNDERWOOD... "Hunting with Canoe and Camera in New Brunswick."

Friday, Aug. 8,
Dr. Selig Hecht............"The Nature of the Sensitivity of Animals to Light."
Monday, Aug. 11.
Dr. Christo A. Dako........"Albanian Rights and Claims to Independence."
Tuesday, Aug. 12,
Dr. R. A. Harper..........."Some Studies of Morphogenesis in Cœnobic Plants."
Thursday, Aug. 14,
Miss Anna Novakova......."New Czecho Slovak State."
Lieut. Peck................"Conditions in Central Europe."

Friday, Aug. 15..
Dr. R. Constantian........."Constantinople."

6. MEMBERS OF THE CORPORATION

Life Members

Allis, Mr. E. P., Jr., Palais Carnoles, Menton, France.
Andrews, Mrs. Gwendolen Foulke, Baltimore, Md.
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Clarke, Prof. S. F., Williamstown, Mass.
Conklin, Prof. Edwin G., Princeton University, Princeton, N. J.
Crane, Mr. C. R., Woods Hole, Mass.
Davis, Major Henry M., Syracuse, N. Y.
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7. CORPORATION MEMBERSHIP LIST

August, 1919

Addison, Dr. W. H. F., University of Pennsylvania Medical School, Philadelphia, Pa.
Adams, Miss A. E., Mount Holyoke College, South Hadley, Mass.
Agersborg, Mr. H. P. K., Hoagland Laboratory, Long Island College Hospital, Brooklyn, N. Y.
Allee, Dr. W. C., Lake Forest College, Lake Forest, Ill.
Allen, Prof. Ezra, Ursinus College, Collegeville, Pa.
Allyn, Miss Harriet M., Hackett Medical College, Canton, China.
Alsberg, Dr. C. S., U. S. Dept. of Agriculture, Washington, D. C.
Altenburg, Dr. Edgar, Rice Institute, Houston, Texas.

Baitsell, Dr. George A., Yale University, New Haven, Conn.
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Baker, Dr. E. H., American Institute of Science, Chicago, Ill.
Baldwin, Dr. F. M., Iowa State College, Ames, Iowa.
Bancroft, Prof. F. W., Aloha Farm, Concord, Calif.
Beckwith, Miss Cora J., Vassar College, Poughkeepsie, N. Y.
Behre, Miss Elinor H., Sophie Newcomb Memorial College, New Orleans, La.
Bigelow, Prof. M. A., Teachers College, Columbia University, New York City.
Bigelow, Prof. R. P., Mass. Institute of Technology, Cambridge, Mass.
Binford, Prof. Raymond, Guilford College, Guilford College, N. C.
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Box, Miss Cora May, University of Cincinnati, Cincinnati, Ohio.
Bowen, Dr. Robert H., Columbia University, New York City, N. Y.

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Harvey, Mrs. E. N., Princeton, N. J.

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HOLMES, PROF. S. J., University of California, Berkeley, Calif.

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HOYT, DR. WILLIAM D., Washington and Lee University, Lexington, Va.

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JEWETT, PROF. J. R., Harvard University, Cambridge, Mass.

JONES, PROF. LYNDS, Oberlin College, Oberlin, Ohio.

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SEXUAL DIMORPHISM IN NEMERTEANS.

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It has been generally assumed by zoölogists that the sexes of the nemerteans are indistinguishable, with the exception of possible size and color differences, without an examination of the gonads. While this is generally true certain facts have been recently brought to light which show, as will be explained below, that in at least one genus the sexes are truly dimorphic. The male in this case not only bears the gonads in an entirely different part of the body than does the female, but is also distinguished by the possession of a pair of large lateral appendages which are apparently used as copulatory organs. The only other instance of an external appendage in nemerteans is the caudal cirrus in *Micrura* and related genera, but this is without relation to sex.

Many cases are known in which both size and color differences distinguish the sexes when mature. Thus it has long been known that in the common *Cerebratulus lacteus* Ver. the males in the breeding season (Coe, '95) are suffused with bright red in the anterior portions of the body and deep red in the intestinal region where the spermaries are located, while the females are much darker, duller red or brownish red, with a grayish tinge except in the anterior portions of the body. This differentiation in color is apparent some two or three months before the sexual products are ready to be discharged, in April, although in the summer and autumn months both sexes are nearly alike, with pinkish white bodies and yellowish or brownish intestinal diverticula. Many other species show somewhat similar differences in color when sexually mature, or in the breeding season of those which live for several years. It seems not improbable that the sexes in every species if carefully observed would likewise be recognizable by color modifications when their sexual products are ripe.

Little evidence is available as to the size factor in relation to sex, but in general the exceptionally large individuals are females.

The third external sexual character, in which a real dimorphism of the sexes occurs, has been found only in a single family living in the deep sea in various parts of the world. And although both males and females of one of these forms have been known for many years the sexes have hitherto been placed in different genera. The form to which reference is made was described by Verrill nearly thirty years ago (1892) as the type of a new family, Nectonemertidæ, the males being placed in the genus *Nectonemertes*. The species was named by him *N. mirabilis*. The most remarkable feature of this new genus was the presence of a pair of lateral appendages, or tentacles, on the sides of the body just back of the head.

A. Sexual Dimorphism in Nectonemertes.

Verrill correctly noted the fact that in *N. mirabilis* the smaller and immature individuals had very short, or rudimentary, tentacles while in the largest specimen these organs attained a length much greater than the diameter of the body. The four specimens which Verrill had for study were collected by the United States Fish Commission Steamer Albatross in the North Atlantic Ocean in the region of the Sargasso Sea, between 37 and 41 degrees north latitude and between 66 and 73 degrees west longitude. The depth of water at the collecting stations varied from 600 to 1,700 fathoms. These specimens and one other from the same locality have recently been fully studied by the writer, and all prove to be males.

The worms of this species have a remarkably fish-like appearance (Figs. 1, 2), with much flattened bodies, with horizontal fins along the posterior half of the body, and a broad terminal tail fin. They are obviously adapted to a pelagic existence, and although there is no positive evidence as to the exact depth at which this particular species lives, yet several other species of the genus are known to live at intermediate depths in the deep oceans, and other forms of a somewhat similar organization have been taken in a closing net at a depth of several hundred fathoms. The epithelium covering the body in all these forms is mostly dislodged during capture, and in one or two species mentioned below the change in pressure between their natural habitat and the surface has caused the rupture of the body walls and the extrusion of certain of the internal organs. The evidence all points to a pelagic life at great depths, that is, from several hundred feet to hundreds of fathoms. That is to say, they are truly bathypelagic organisms.

In the same collection and from the same locality were two specimens which Verrill ('92) described as belonging to another genus and species, *Hyalonemertes atlantica*, with characteristics very similar to those of *Nectomertes*, but which lacked any trace of tentacles. By a curious error, evidently made in transcribing his notes, the ovaries of these females were incorrectly described by Verrill as the gonads of *Nectonemertes*. But for this confusion it is possible that Verrill would have himself recognized the sexual dimorphism of this form.

Since Verrill's original description of *Nectonemertes* several other species of the genus have been described from as many different localities. One species, *N. pelagica* Cravens and Heath ('06), occurs off the coast of California. Another, *N. japonica* Foshay ('12), lives in Japanese waters. Three others, *N. grimaldii* Joubin ('04) from west of the Azores, *N. chavesi* Joubin ('06) from south of the Azores, *N. lobata* Joubin ('06) from near the Azores, were each described from the superficial study of a single specimen. As the specific distinctions rest largely on the peculiarities of the tentacles, Joubin's three supposed species may eventually prove to represent merely growth stages of but a single species.

The tentacles of *N. chavesi* were represented merely by blunt papillæ or short tubercles (Fig. 11, *t*) in the only specimen studied by Joubin. The photograph shows indication of minute cephalic spermaries (Fig. 11, *sp*), although Joubin did not recognize them as such. Both these characters indicate an immature male individual. In *N. grimaldii*, on the other hand, both tentacles and cephalic spermaries are well developed (Fig. 9), indicative of a mature male. Joubin correctly describes the external appearance of the spermaries, but suspected that they might be organs of a glandular or sensory nature. In the single specimen of *N. lobata* available for study by Joubin, one of the tentacles was prolonged into a slender filament. This presumably represents merely a relaxed condition of the tentacle, for Verrill ('92) describes one or two of his specimens of *N. mirabilis* as having similar filamentous terminal portions, while in others the tentacles were contracted into relatively shorter and thicker appendages, as shown in Fig. 1.

Bürger ('09) studied and described anatomically one specimen of *Nectonemertes* from off the coast of Jumba, French Congo, which he identified as *N. mirabilis.* Finally Brinkmann ('16) has described *N. minima* from three males and one female, also collected off the west coast of Africa.

In *N. minima* the tentacles are similar to those of *N. mirabilis*, as are also those of *N. pelagica* (Fig. 19). All of these descriptions, however, were made from dead or preserved specimens. The appearance of the living animal in one of these forms, *N. mirabilis*, is shown in Fig. 18. In life the tentacles are much longer and more slender than they appear in the preserved specimen. A comparison of Figs. 18 and 19, although representing different species, will indicate the change in form which takes place upon the death of the animal.

Thus far about twenty-four specimens of the seven species enumerated above have been described as having tentacles, and all were males. Verrill, Joubin, and Bürger recognized the close structural similarity between *Nectonemertes* and *Hyalonemertes*, but Brinkmann ('12) was the first to suggest that they might represent dimorphic forms of a single species. As the name given to the male, *Nectonemertes*, has priority over that of the female, *Hyalonemertes* in Verrill's work ('92), Brinkmann correctly refers his species to the former genus.

It has been mentioned that more male individuals than females of the various species of this genus have been studied. For example, Cravens and Heath found only males in their five specimens of *N. pelagica*, while Foshay was sent six specimens of *N. japonica*, which were likewise all males. Three reasons for this suggest themselves. First, the tentacles of the male are presumably adapted for clasping and holding the female. As a result of their clinging instinct the worms hold fast to any small foreign object such as a fishing line or bait or the mesh of a net which may be lowered to their habitat. The males are thereby drawn to the surface and collected. Second, the very conspicuous appendages of the males would direct attention to them as unusual objects and they would be carefully preserved by the inexperienced collector—and some of the specimens are known to have been obtained in this way—while the closer resemblance of the females to other common species of nemerteans would seem to give them a lesser importance. And, finally, it seems very possible from such meager descriptions as Joubin ('06) gives of six supposedly new species of *Planktonemertes* that one or more of them may actually be females of species of *Nectonemertes*, for they come from the same general region of the North Atlantic Ocean; that is, from stations west of the Azores only a few degrees apart. With these and perhaps other considerations in mind it is not surprising that more males than females of the various species are known.

Gonads.—The two sexes in *Nectonemertes* are distinguished not only by the tentacles characteristic of the males but also by the position and appearance of the gonads. The spermaries of the male (Fig. 1) are limited to the head region, while the ovaries of the female are situated in their primitive positions between the intestinal diverticula along the sides of the body (Fig. 2). The general appearance of the two sexes thereby becomes so strikingly different that their original separation into different genera is hardly surprising.

Tentacles.—Microscopic study of the tentacles of *Nectonemertes* shows that they represent slender outgrowths of the body walls; the integumentary, basement, and muscular layers being the same in both. The muscular layers, however, assume a direction at right angles to those of the body, and are of far greater thickness. The central part of the appendage is filled with an extension of the body parenchyma, with blood lacunæ of large size, and two very large nerves which originate from several branches of the lateral nerve cord at points near the base of the tentacle.

The structure of the tentacles indicates clearly that these organs are capable of a high degree of muscular contraction. The filamentous character of their terminal portions when well developed suggests that they are not simply locomotor organs, but that they are specially adapted for grasping. The highly developed horizontal and caudal fins show that the animals are free swimming. When it is remembered further that the tentacles reach their full size only at the time of sexual maturity, the conclusion seems reasonable that they are then used for grasping the female and clinging to her during the act of insemination, as Brinkmann ('12) has suggested. Associated with these organs are spermaries with highly muscular walls (Fig. 8) opening by sperm ducts leading to the ventral surface of the head directly anterior to the tentacles (Figs. 1, 8). The number of such spermaries is limited to from twenty to thirty. The female also differs from most littoral nemerteans, but agrees with those of other bathypelagic species in having relatively few pairs of ovaries along the sides of the body (Fig. 2). And each ovary produces but one or two eggs of relatively enormous size as compared with those of most littoral species.

The reduction in the number of spermatozoa to a small fraction of the number found in a worm of a littoral species of similar size, and a corresponding reduction in the number of ova produced by the female, has evidently necessitated a conservation of the genital products by adaptations for securing the fertilization of the maximum proportion of the eggs produced.

Hence the advantage of pairing instincts and organs for accomplishing this result in place of the more primitive and wasteful processes which obtain in the littoral forms. These latter, it will be remembered, in many cases discharge an enormous number of spermatozoa and minute eggs into the water about them, with relatively small chances of any particular egg producing an embryo. In some of the smaller forms, on the other hand (as *Amphiporus*), there is a kind of pairing in which the two sexually mature individuals of opposite sex place their bodies side by side and the genital products of both are discharged together in a mass of mucus secreted by the integument of the two worms. The littoral forms moreover live in a comparatively limited environment and thus have a great advantage in this respect over these bathypelagic species, the habitat of which may extend over hundreds of miles of ocean, with a vertical range of several hundred fathoms. Hence, the advantage of organs by which the sexes when mature may be held together until insemination occurs. For it may be assumed that fertilization of the eggs takes place within the body of the female, as in several well-known littoral and terrestrial species. The early formation of the oviducts indicates that this is probable, although we have as yet no direct evidence that it actually occurs. Moreover, it may be not unreasonable to suspect the possibility of embryonic development within the mother's body, which in the known viviparous forms is often associated with eggs of unusual size, as in *Geonemertes*, for example (Coe, '04).

The position of the sperm ducts on the ventral surface of the head just anterior to the tentacles, where they could be most effectually pressed against the open oviducts of the female, is a further indication that internal fertilization takes place. And finally, the highly muscular walls of the spermaries (Fig. 8), which are found only in bathypelagic species, doubtless serve for the forcible ejection of the spermatozoa through the slender sperm ducts which open at the summit of small extensible papillæ. The weakness of the musculature of the body walls is thus compensated for by the special musculature of the spermary. In *Bathynectes murrayi* Brinkmann finds similar muscular spermaries connected with slender sperm ducts which extend as penes far beyond the surface of the body (Figs. 15, 16, *pe*), as described below. Such a condition indicates that the sperm ducts may be actually inserted into the open oviducts of the female. Then by the contraction of the musculature of the spermary some of the spermatozoa contained in the latter would be forced into direct contact with the one or two large ova which each ovary contains. The smaller genital papillæ of *Nectonemertes* may function in a similar manner.

In littoral and terrestrial species where fertilization takes place within the body of the female the two worms, after coming in contact, secrete a great abundance of mucus which surrounds the two animals as a sheath and prevents the escape of the spermatozoa after they have been discharged by the male. These bathypelagic species, on the other hand, are but poorly supplied with mucus secreting glands and hence appear to require the actual introduction of the spermatozoa into the oviducts in order that fertilization may be assured.

B. SEXUAL DIFFERENTIATION IN OTHER BATHYPELAGIC NEMERTEANS.

The distinction between sexual differentiation and sexual dimorphism should closely be borne in mind. As commonly used the former term has reference to those characteristics which distinguish the sexes in a form in which both sexes are easily recognized as belonging to the same species, while the latter implies morphological features so profound that the two sexes might appear to belong to different species or even larger groups. Among the nemerteans known at the present time only *Nectonemertes*, and possibly *Balænanemertes*, belong to the latter category.

It may be worth while to consider in this connection a few of the many cases of sexual differentiation which lead toward the true sexual dimorphism described above for *Nectonemertes*. In many species of nemerteans there can be no question as to sexual differentiation, for all individuals are hermaphroditic and to some extent protandric, as in the fresh-water *Stichostemma* and the terrestrial *Geonemertes* (Coe, '04). Here the young and small individuals function as males. A period of growth commonly follows the discharge of most of their spermatozoa, when they assume the characteristics of the female. In some cases both spermatozoa and ova develop at the same time. Some of these forms are viviparous, but there are also several cases of viviparity known among littoral species which are neither hermaphroditic nor protandric.

The primitive condition of the reproductive organs in the nemerteans, as is well known, is that of separate sexes, in both of which the gonads occupy similar positions along each side of the body throughout almost its entire length back of the head. The gonads are usually in pairs, alternating regularly with the intestinal lobes, or diverticula. The number of such gonads may be many thousands in a large worm, and each ovary as well as each spermary produces a large number of gametes. It is estimated that a large female Cerebratulus, which may reach a length of two meters or more, produces upwards of one hundred millions of eggs in one season. These are all discharged within the space of a few days. And in some littoral species no larger than *Nectonemertes* the number of eggs produced is at least a hundred times greater than in the latter.

In all the bathypelagic forms the gonads of both sexes are reduced in number and size to but a small fraction of those of littoral species. A comparison of the forms hitherto described with respect to these structures will show a graded series leading to the extreme condition found in *Paradinonemertes* in which the male is provided with but two pairs of spermaries (Fig. 17 *sp*).

In all the bathypelagic forms moreover the spermaries are limited to the anterior regions of the body, and indeed in most species are far removed from their primitive position to the region of the head. In a few forms they are even limited to the area in front of the brain, where they are crowded into a dense cluster on each side of the head, with their sperm ducts opening near the lateral borders of the mouth.

In the female the ovaries remain in all species in their primitive interdiverticular positions, but as the number of intestinal diverticula becomes reduced the ovaries suffer a corresponding reduction. The extreme limit in the species known at present appears to be four or five pairs, which number occurs typically in *Pelagonemertes rollestoni*.

1. *Planktonemertes*.—In *Planktonemertes* occurs one of the first steps in the process of reduction. Strangely enough, however, of the several species of this genus thus far described only the females are known with the exception of the single specimen of *P. alberti*, in which Joubin (1906) clearly shows the two groups of spermaries behind the brain. This investigator did not, however, suspect their true nature. Fortunately an exceptionally well preserved specimen of *P. agassizii* has recently come into my hands which proves to be a sexually mature male. This was collected by the U. S. Fish Commission Steamer Albatross from near the equator in longitude 81° West; that is, off the coast of Ecuador. A study of this specimen reveals nine rounded spermaries on one side and eleven on the other, situated immediately back of the brain, medially to the lateral nerves, and laterally to the pylorus (Fig. 3, *sp*). These spermaries presumably represent the anterior interdiverticular gonads

of littoral species, although they lie far anterior to the intestine proper. Yet in this and related forms the intestinal diverticula extend far forward, even in front of the brain in many cases. It is evident that in their embryonic development these diverticula actually grow anteriorly from their primitive lateral positions, and it is quite conceivable that they carry with them the rudiments of the gonads and that only those gonads which are thus carried forward develop into spermaries. Serious objections to this hypothesis are, first, that the number of spermaries bears no relation to the number of cephalic diverticula present in the adult, and, second, even with this hypothesis a certain amount of independent migration would be necessary to give the spermaries their definite localization.

The number of intestinal diverticula in this species is commonly between thirty and fifty and the ovaries retain their primitive positions between them (Fig. 4). One of the females available for study, although of large size, had the ovaries in a very early stage of development. In this case these organs alternated regularly with the intestinal diverticula except toward the posterior end of the body. But in the specimens with mature ovaries the number was much less than that of the diverticula, ranging from about fifteen to twenty pairs (Fig. 4). Evidently some of the original ovaries have failed to develop or have been absorbed by the body tissues.

This process if carried still farther would result in a condition similar to that which Bürger ('06) found in a related species, *P. woodworthi*, in which there are only seven or eight pairs of ovaries. Bürger also found in a specimen which he erroneously identifies as *P. agassizii* "numerous" very young ovaries which alternate regularly with the diverticula in the middle portions of the body, although he does not state the precise number. Brinkmann ('16) studied a single female of *P. vanhöffeni* which had just discharged the eggs from fourteen pairs of ovaries.

The conditions in the females of all these species are thus quite in harmony with the account given above for *P. agassizii*, but only in the latter species has the male been discovered.

2. *Balænanemertes.*—Bürger ('09) describes from a single specimen a new genus which in his opinion forms a connecting link

between the nemerteans with and those without tentacular appendages. This species, *Balænanemertes chuni*, was taken in the Indian Ocean, in a region where the depth of water was 2,500 meters. This specimen, although a sexually mature male with cephalic gonads, was provided with a pair of short sickle-shaped lateral processes, or papillæ (Fig. 10, *t*) closely resembling the rudimentary tentacles in the young individuals of *Nectonemertes mirabilis*. It is by no means certain, however, that these processes might not have developed into longer tentacles if the individual had lived until the spermatozoa were quite ready to be discharged. They would doubtless have been much more conspicuous in life than they appear in the preserved specimen, for the tentacles of Nectonemertes are such highly distensible organs that they suffer enormous contraction upon preservation, as is indicated in figures 18 and 19.

There are five pairs of spermaries in this species. These are situated immediately behind the brain. Each gonad opens by a short sperm duct which leads to the summit of a blunt or rounded papilla (Fig. 10, *sp*) on the lateral border of the head. Female is still unknown.

3. *Bathynectes*.—In *Bathynectes murrayi* Brinkmann ('12) finds a remarkable modification of the reproductive organs in the male. In this bathypelagic nemertean from the North Atlantic the spermaries are provided with muscular walls as in *Nectonemertes*, and the sperm ducts, instead of ending in minute papillæ on the surface of the head as in all other species, are prolonged in some individuals into slender muscular penes (Figs. 15, 16, *pe*).

There are from five to seven pairs of these organs on the ventral side of the head, corresponding to the same number of cephalic spermaries (Figs. 15, 16). That the penis is actually an outgrowth from the body walls is shown by the fact that its walls, like those of the body, consist of basement layer and circular and longitudinal musculatures. The organ is evidently capable of muscular contraction and may be considered a true copulatory organ.

In several of the specimens collected the penes were torn from their insertions in the body walls, and Brinkmann offers the suggestion that they may have been inserted into the ovaries of the females and held there to serve as spermatophores.

The female of this species has about 25 pairs of typical interdiverticular ovaries.

4. *Paradinonemertes.*—In *Paradinonemertes drygalaskii* Brinkmann ('16), of which two specimens are thus far known, the spermaries have suffered a still further reduction in number, being limited to two pairs of gonads situated immediately behind the brain and between the stomach and the lateral nerve cords (Fig. 17, *sp*). This is the extreme limit of reduction at present known among the nemerteans. These two pairs occupy the same position in the body as do the two anterior pairs in *Planktonemertes*, and are apparently homologous with them. The female of this species is still unknown.

5. *Pelagonemertes.*—It is in the genus *Pelagonemertes* that the spermaries show the greatest deviation from the primitive arrangement. In *P. brinkmanni*, a new species collected in the northwest Pacific Ocean, there are five to seven egg-shaped spermaries in a single cluster (Figs. 5, 7, *sp.*) on each side of the head in front of the brain. These open by a group of small genital papillæ situated ventrally on the antero-lateral border of each side of the head (Fig. 7, *gp.*).

In some cases the contraction of the tissues during the capture and preservation of the worms has ruptured the delicate cephalic walls and forced the spermaries quite outside of the body. They then appear as if they were external appendages on the anterior margins of the head.

The ovaries likewise suffer great reduction in number as compared with the forms previously considered, there being usually only six pairs of these gonads (Fig. 6, *ov*). Occasionally the number may be increased to seven or eight on one or both sides. The immature ovary contains as many as four to six or more small eggs, but as development proceeds most of these are engulfed as food material for the one, two, or occasionally three very large ova which reach maturity. The total number of eggs which one of these worms produces in a season is therefore hardly more than twenty to thirty.

In *Pelagonmertes rollestoni* Bürger ('09) finds likewise five or six pairs of spermaries (Fig. 12, *sp*) closely grouped on each side in front of the brain. The sperm ducts leading from each group open close together on the ventral surface of the head and close beside the rhynchodeal opening, somewhat as described above for *P. brinkmanni*.

In *Pelagonemertes mosleyi* there are seven or eight pairs of ovaries, while in *P. rollestoni* the number may sometimes be reduced to four or five (Fig. 13, *ov*). As each of these ovaries produces but one very large egg, or occasionally two or three, the total number of eggs which mature in a season is hardly more than ten to twenty under the most favorable conditions. This number is in marked contrast to the hundred million which a single large female of one of the littoral species discharges in the space of a few days. The few however are so abundantly provided with nourishment that it is probable that the entire embryonic development is completed without the necessity of securing additional food. Since the oviducts are formed long before the maturity of the ova, it seems reasonable to assume that fertilization is internal, and it appears quite possible that the entire embryonic history is completed within the parent's body.

Summary.—The foregoing brief survey of the structural modifications of the body, and especially of the organs concerned in reproduction, which have recently been discovered in the pelagic nemerteans shows that the series may be looked upon as representing possible steps in an evolutionary series leading from the primitive condition of the well-known littoral forms to the highly specialized adaptations of organisms living as a sparse population in the vast areas of the open oceans. These modifications all tend toward a reduction in the number of gametes produced, correlated with increasing provisions for insuring fertilization and the survival of the relatively few offspring produced, and leading eventually to a true sexual dimorphism.

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Explanation of Plate I.

Fig. 1. *Nectonermertes mirabilis* Verrill. Mature male, with well-developed tentacles and upwards of fifteen pairs of spermaries (*sp*), situated on the ventro-lateral borders of the head; *dg*, dorsal ganglion of brain; *ln*, lateral nerve; *cv*, cephalic blood vessel; *mv*, median blood vessel; *lv*, lateral blood vessel. × 6.

Fig. 2. Female of same species, with from sixteen to nineteen ovaries (*ov*) on each side of body. Each ovary contains but one large ovum, or sometimes two; *br*, brain; *ps*, proboscis sheath. × 6.

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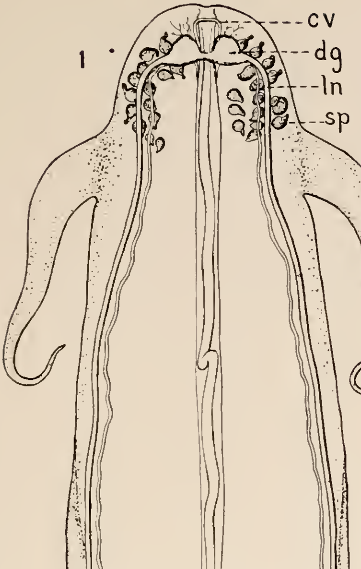
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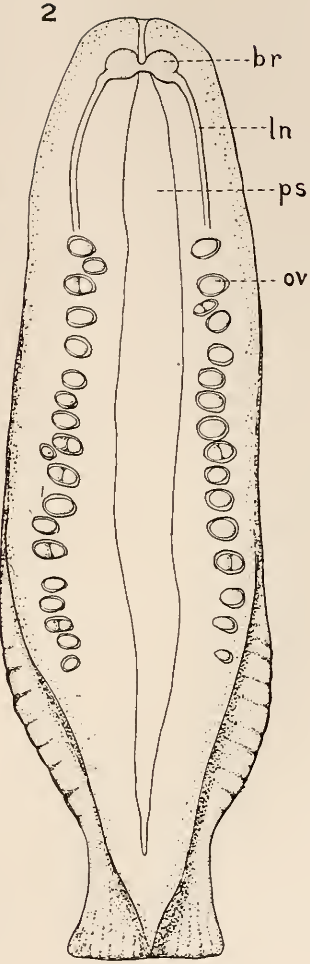

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Explanation of Plate II.

Fig. 3. *Planktonemertes agassizii* Woodworth. Outline of body of mature male with nine spermaries (*sp*) opening on the ventral surface on one side of the body immediately back of the brain and eleven on the other side; *r*, rhynchodeum; *br*, brain; *ln*, lateral nerve; *ps*, proboscis sheath. × 6.

Fig. 4. Outline of body of female of same species, showing the ovaries (*ov*), each with one or occasionally two large ova, situated beneath the lateral nerves on each side of the body. × 6.

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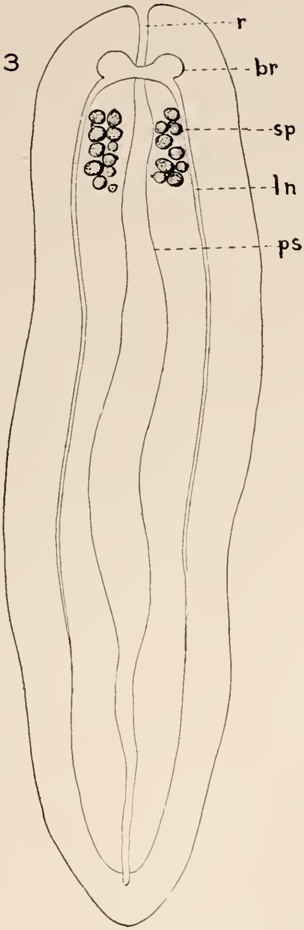

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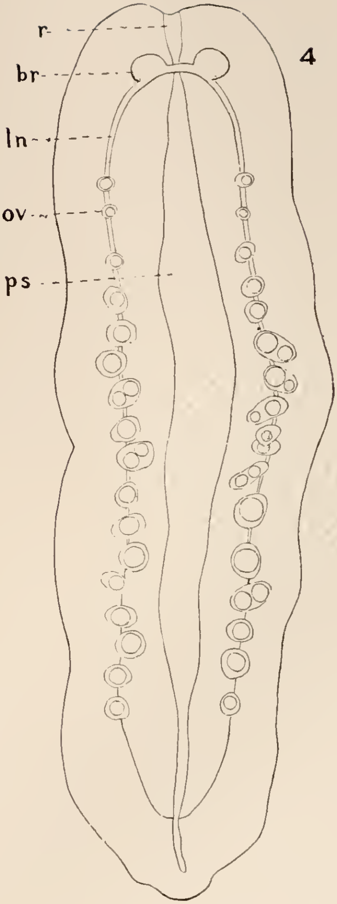

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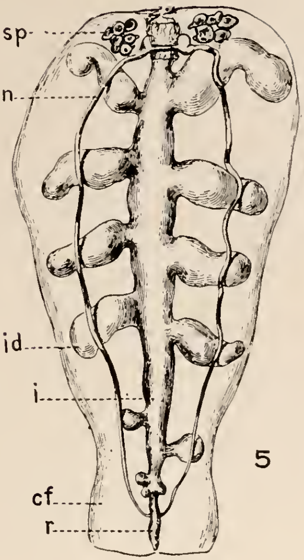
Explanation of Plate III.

Fig. 5. *Pelagonermertes brinkmanni*, new species. Outline of body of male, showing the spermaries (*sp*) situated on the anterior margin of the head. The digestive and nervous systems are also shown; *i*, intestine; *id*, intestinal diverticulum; *r*, rectum; *ln*, lateral nerve; *cf*, caudal fin. × 6.

Fig. 6. Outline of body of female of same species, showing the six pairs of ovaries (*ov*), each with usually a single large egg. × 6.

Fig. 7. Anterior portion of body of same species, showing the five ovate spermaries (*sp*) which open on the anterior border of the side of the head; the genital openings here lie close together on blunt papillæ, but in some cases are more widely separated, due to different contraction of the adjacent tissues; *m*, mouth; *st*, stomach; *id*, intestinal diverticulum; *py*, pylorus. × 12.

Fig. 8. *Nectonemertes mirabilis*. A mature spermary, showing its thick muscular layer; *sv*, thin-walled seminal vesicle; *gp*, genital papilla. × 60.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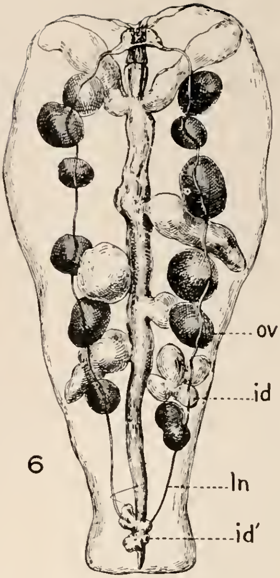

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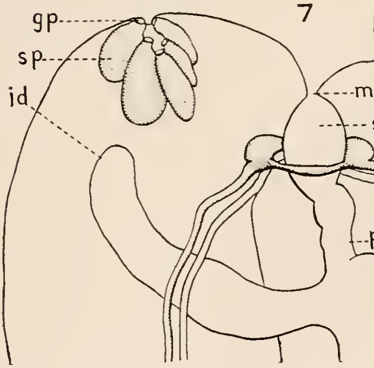
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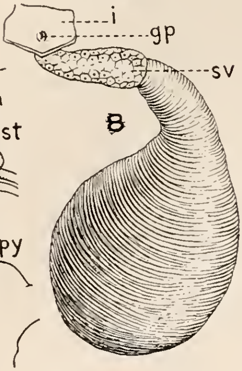

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EXPLANATION OF PLATE IV.

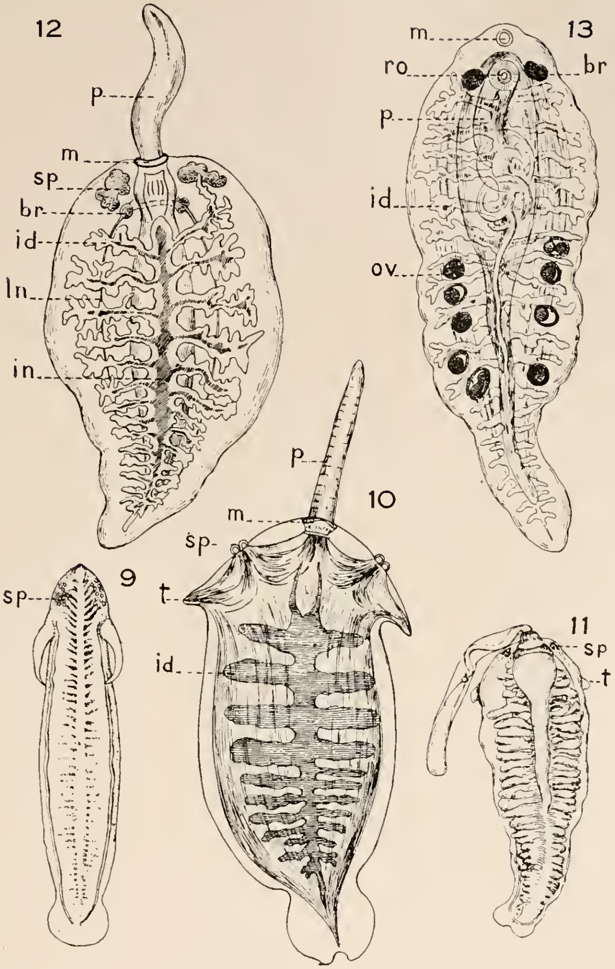
FIG. 9. *Nectonemertes grimaldii* Joubin. Male. Sketch of living animal, showing tentacles of medium size. × 1½. (After Joubin, '06.)

FIG. 10. *Balænanemertes chuni* Bürger. Sketch of male after preservation, showing the rudimentary tentacles (*t*) and the genital papillæ (*sp*) on which the sperm ducts open. × 8. (After Bürger, '09).

FIG. 11. *Nectonemertes chavesi* Joubin. From photograph of male with rudimentary tentacles (*t*). The spermaries (*sp*) are indicated on each side of the head. × 5. (After Joubin '06.)

FIG. 12. *Pelagonemertes rollestoni* Moseley. Adult male, with a single group of five or six closely placed spermaries (*sp*) on each side of the head; *p*, partially extruded proboscis; *m*, mouth; *br*, brain; *in*, intestine; *id*, intestinal diverticulum; *ln*, lateral nerve. × 3. (After Bürger, '09.)

FIG. 13. *P. rollestoni.* Female, showing the four or five ovaries (*ov*) on each side, each with from one to three ova; *ro*, rhynchodeal opening; other lettering as in figure 12. × 8. (After Bürger, '09.)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Explanation of Plate V.

Fig. 14. *Nectonemertes minima.* Outline of head, showing position of the spermaries (*sp*) with their long genital ducts (*sd*) leading to the ventro-lateral surfaces of head; *m*, mouth; *br*, brain; *t*, base of tentacle. × 24. (After Brinkmann, '16.)

Fig. 15. *Bathynectes murrayi.* Outline of anterior portion of body, showing the five pairs of protruding genital ducts (penes, *pe*); *m*, mouth. × 6. (After Brinkmann, '12.)

Fig. 16. Same species. Outline of three of the muscular walled spermaries (*sp*), showing the genital ducts (*pe*) protruding from the body walls (*bw*). × 12. (After Brinkmann, '12.)

Fig. 17. *Paradinonemertes drygalskii.* Outline of anterior portion of body of male, showing the two pairs of spermaries (*sp*) behind the brain (*br*); *ro*, rhynchodeum; *m*, mouth; *id*, intestinal diverticulum. × 12. (After Brinkmann, '16.)

Fig. 18. *Nectonemertes mirabilis.* Sketch of living, sexually mature male with fully developed tentacles. Length of body after preservation 19 mm. × 4. (After Bürger, '09.)

Fig. 19. *N. pelagica.* Outline of body of sexually mature male. × 6. (After Cravens and Heath, '06.)

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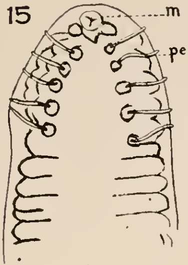

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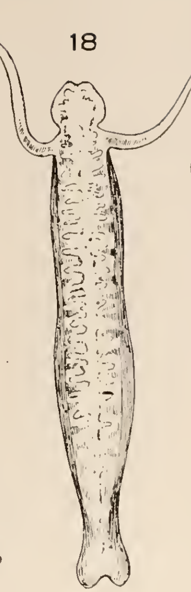

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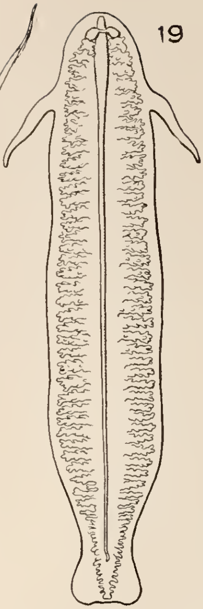

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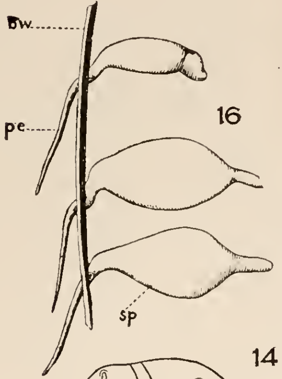

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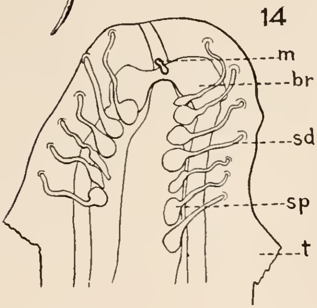

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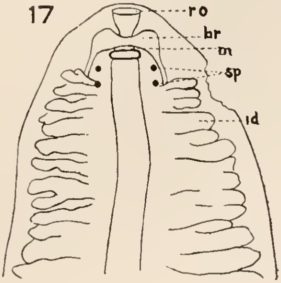

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A STUDY OF THE COMBINED ACTION OF X-RAYS AND OF VITAL STAINS UPON PARAMŒCIA.

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It has been definitely recognized that X-rays exercise injurious effects upon both vegetable and animal forms of life and, moreover, that the adult tissues possess a variable absorption capacity for this form of energy. On the other hand, experiments conducted for the purpose of ascertaining the nature of the reactions of bacteria to X-rays have generally proved negative. In marked contrast to seeming bacterial immunity, unicellular organisms show a marked degree of susceptibility.

During the spring and summer of 1916, through the courtesy of the Research Laboratory of the General Electric Company at Schenectady, the author was enabled to make use of X-ray energy in a prosecution of the study of the action of vital stains upon various forms of animal life. These experiments were begun with paramœcia. The investigation comprised, first, the study of the action of vital stains upon these forms of life; secondly, of the effect of X-rays acting alone; and lastly, a study of the reaction to X-ray energy while under the influence of these vital stains. The stains used for this purpose were Nilblau sulfat (Gruebler), Alizarinblau (S), Trypanblau, Isaminblau, Nilblau chlorhydrat, Trypanrot, Dahlia (Gruebler), Neutral red, Anilin red (Sudan III. oil), Janus green (Eimer and Amend), Methylene blue (B.X.) (Merck). These stains were in the form of dry powders. Solutions were prepared with tap water. It had been previously ascertained that this tap water contained no substances injurious to the organisms either at the time of raying or thereafter. The solutions of the stains were freshly prepared for each experiment. In the majority of instances a stock solution consisting of 0.001 gm. of stain to 200 c.c. of water was used, a subsequent dilution of this stain being made before each experiment. This stock solution was not kept for a period longer than twenty-four hours. Cultures of the paramœcia were added to graded dilutions of the stain with the object of ascertaining the maximum degree of concentration compatible with the life of the organism. The absorption of the stain by the organism was demonstrable through the appearance of stained globules within the body. It was ascertained early in the experiment that this absorption would progress, if undisturbed, to such a degree that the death of the organism resulted. When this phenomenon was about to ensue the motility of the organism became very sluggish, finally ceasing altogether. The elongated, elliptical body-shape was replaced by a spherical form, while the nucleus quickly became deeply stained. It was found not to be possible to bring about a staining reaction of the nucleus without causing the death of the organism. Indeed, the latter became a safe index of over-absorption of the stain.

For the purpose of testing out the absorption capacity for the stain compatible with the life of the organism, 0.5 c.c. cultures of the paramœcia were placed in ordinary watch glasses, to which were added varying amounts of the solutions of the stains. In the preliminary experiments these proportions ranged from two volumes of culture to twelve volumes of stain, to eight volumes of culture to two of stain. The cultures were then carefully attended during the succeeding days with the view of providing sufficient oxygen and to prevent evaporation. A reference to a typical experiment of May 31, 1916, A.M., will be sufficient to give an exact idea of this procedure. For this experiment a stock solution of neutral red in the strength of 0.001 gm. of the dry stain of 200 c.c. water was used.

With such stains as methylene blue and dahlia the absorption capacity of the organisms was so great that it was difficult even in greatly diluted solutions to prevent the staining of the nucleus. With trypanblau, neutral red, and trypan red, on the other hand, the capacity of the organisms for the stain seemed to have a more definite though not absolutely fixed limit. In these instances it was ascertained that, when the cell-body had taken up its maximum carrying amount of the stain, not injurious to the activity of the organism, subsequent immersion over a relatively long period of time in a very greatly diluted solution

Experiment May 31, 1916, A.M.	Observations of June 1, 1916, A.M.	Observations of June 2, 1916, A.M.
Series No. 1, Culture 2 parts (by volume), Stain 8 parts (by volume)	Series No. 1, paramœcia spherical in shape, motionless, nucleus stained.	Series No. 1, paramœcia spherical in shape, motionless, nucleus stained.
Series No. 2, Culture 2 parts (by volume), Stain 7 parts (by volume)	Series No. 1, paramœcia spherical in shape, motionless, nucleus stained.	Series No. 1, paramœcia spherical in shape, motionless, nucleus stained.
Series No. 3, Culture 2 parts (by volume), Stain 6 parts (by volume)	Series No. 1, paramœcia spherical in shape, motionless, nucleus stained.	Series No. 1, paramœcia spherical in shape, motionless, nucleus stained.
Series No. 4, Culture 2 parts (by volume), Stain 5 parts (by volume)	Series No. 1, paramœcia spherical in shape, motionless, nucleus stained.	Series No. 1, paramœcia spherical in shape, motionless, nucleus stained.
Series No. 5, Culture 2 parts (by volume), Stain 4 parts (by volume)	Series No. 1, paramœcia spherical in shape, motionless, nucleus stained.	Series No. 1, paramœcia spherical in shape, motionless, nucleus stained.
Series No. 6, Culture 2 parts (by volume), Stain 2 parts (by volume)	Series No. 6, paramœcia sluggishly active, few motionless with stained nuclei.	Series No. 1, paramœcia spherical in shape, motionless, nucleus stained.
Series No. 7, Culture 3 parts (by volume), Stain 2 parts (by volume)	Series No. 7, all active.	Series No. 7, all active.
Series No. 8, Culture 5 parts (by volume), Stain 2 parts (by volume)	Series No. 7, all active.	Series No. 7, all active.

of the stain did not result apparently in a further absorption. The experiments demonstrated, moreover, that the various stains possessed a differing degree of toxicity for these organisms similar to that which had been noted by Lewis in his study of the action of vital stains on bacteria.

Practically it was found to be more convenient to stain organisms for a period of from one and a half to two hours in a solution of a degree of concentration somewhat greater than that which could be tolerated without injury for a period of twelve hours. At the expiration of this staining period the organisms were transferred to the diluent tap water in the proportion of ten volumes of the water to one volume of the organism and stain and studied during the succeeding days. Through an empirical process of experimentation the approximate degree of concentration tolerated by the organism followed by a dilution as above was ascertained. In the instance of trypanblau, trypan red, isamine blau, and neutral red, this was found to be eight volumes of the water solution of the stain to two of the culture. The stock solution contained 0.001 gm. of the dry stain to 200 c.c. water. Staining of the paramœcia cultures from one and one half to two hours at the room temperature brought about the appearance within one half hour of a deep globular coloration of the cytoplasm of the organism. The subsequent dilution of this stained culture, through the addition of ten volumes of water to one of the culture, prevented subsequent injury, so that the vitality of the organisms remained unimpaired for a period of eight days. The nucleus did not become stained nor was the motility lessened. Ordinarily, the specimens were not studied for a period longer than eight days. With a few, however, observation at the expiration of fourteen days demonstrated still actively motile paramœcia with unstained nuclei. Beyond these limits no effort was made to ascertain either the varying degrees of rapidity of absorption for the different stains or the exact limit of absorptive capacity of the organisms for any particular stain. The dilutions above-mentioned were arbitrarily selected, since with these as a constant, the most marked reactions to X-ray energy were obtained.

The next step in the experimentation consisted of a determination of the amount of X-ray energy necessary to cause the death of unstained organisms. The source of energy was a special Coolidge tube actuated by 35 K.V. with a current strength of 2.0 milliampere and cooled by an airblast. Since the total diameter of the tube was only 7.0 cm. the paramœcia cultures could be placed in ordinary watch glasses at a distance of 8.5 cm. from the center of the target. They were protected from the heat rays arising from the tube by means of three spaced-layers of carbon paper. Numerous experiments showed that there was hardly any loss through evaporation during the raying.

A quotation from the notes will illustrate the general experimental results observed. On the morning of May 5, 1916, a series (*A*) of six cultures, each consisting of numerous unstained paramœcia, was rayed under the conditions mentioned above during a continuous period of thirty minutes. At the expiration of this exposure the motility of the organisms had become appreciably slower. No changes, however, could be observed either in the character of the cytoplasm or of the nucleus under a magnification of 400 diameters. On May 7, 1916, the observation was made at 2:30 P.M. that a few of these paramœcia were still active though they had lost a great deal of the rapidity of action of their cilia. The majority were motionless and rested upon the bottom of the glass. These had assumed a spherical shape, while in them there could be detected unmistakable evidences of degeneration of both nuclear and cytoplasmic material. In the unstained specimens these facts were demonstrated through a loss of their translucent character and by the granular appearance of the protoplasm. Series *B* consisted of six cultures of paramœcia which were rayed under conditions similar to those of series *A* for a period of twenty-six consecutive minutes. At the termination of the exposure all of the paramœcia were vigorously active. There was no indication of the slowing down of ciliary motion. A study of the specimens forty-eight hours thereafter demonstrated a still vigorous motility. No dead paramœcia could be found.

By this method of varying the length of the exposure to the X-ray energy, the other conditions of the experiment remaining constant, it was ascertained that continuous raying during a period varying from thirty to forty minutes was necessary to bring about, upon the day following the raying, a complete sessation of motility and unmistakable evidences of degeneration. It was not possible, however, to cause protoplasmic changes immediately following the raying in either the nucleus or the cytoplasm. These could be made out first only after a twenty-four hour interval after the raying had elapsed. Beyond this no further inquiry was made to ascertain the cytological cause of cessation of motility. Similarly through repeated experimentation, it was found that exposures of from ten to twenty minutes duration exercised no effect whatever, either immediately or subsequently, upon either the motility or the vitality of the organisms. A study of these rayed organisms was not conducted, however, throughout a period greater than eight days after the raying.

The next and final step in the experiment consisted of a study of the action of the rays upon stained cultures of paramœcia. The staining of the organisms was carried out according to the method which I have detailed above. The energy utilized was that which has also been given above. A reference to the experiment notes will give an idea of the results obtained.

On May 10, 1916, series *C*, consisting of six paramœcium cultures, was rayed beginning at 2:00 P.M., each for a period of 12.5 consecutive minutes. These cultures had previously been stained for 105 minutes in a trypanblau solution of the strength 0.001 gm. of the dry stain to 220 c.c. tap water, of which mixture four volumes were added to one volume of the culture. The staining was begun at 11:15 A.M., and the raying at 2:00 P.M. At the expiration of the raying period, all of the organisms were very sluggish in action. At the expiration of forty-eight hours all were dead. Controls to these experiments were conducted in which the unstained paramœcia were rayed for an equal time, and paramœcia were stained during an equal time. In both instances the organisms were unaffected. In one series (*B*) the unstained paramœcia were rayed for a period of 12.5 consecutive minutes and in *C* during a period of thirty consecutive minutes. Forty-eight hours afterwards the unstained cultures were still alive and vigorous but in series *C* most of the paramœcia were dead, a few, however, remaining sluggishly active.

By a continuation of the experiment along this general line, through increasing the concentration of the stain, it was found possible to cause the death of the organisms with an exposure varying from 2.5 to 5 minutes. A reference to the experiment notes of May 18, 1916, verifies this fact. On this day series *A*, consisting of six cultures of paramœcia, was stained with trypanblau for two hours (stock solution 0.001 gms. to 200 c.c. tap water, diluted in the proportion of 2 volumes of culture to 8 of stain). These were then rayed for five consecutive minutes, beginning at 10:42 A.M. At 11:00 A.M. a few of the paramœcia were observed to be active though this activity was considerably slowed. The majority, however, were motionless. Forty-eight hours later a still greater number of paramœcia were dead. The nucleus had assumed a dark bluish color, the cytoplasm had become opaque and deeply blue in color, and the organism had assumed a spherical outline. A few, however, were still sluggishly active. On the succeeding day, these also died. The control specimens which were treated to the stain in the same manner, but which were unrayed, were still alive on May 26, eight days afterward. On May 22, six other cultures, series *F*, were rayed 2.5 consecutive minutes after treatment with trypanblau stain prepared as in series *A*. They had been subjected to this stain for two hours previous to the raying. Very few of these paramœcia were alive on the succeeding day. The majority demonstrated a stained nucleus, spherical outline, and loss of ciliary activity. Controls to this series, however, some of which were rayed unstained for a period of ten minutes, and others stained from 11:15 A.M. to 1:41 P.M., but unrayed, were still alive seven days later, and, judging by their activity, had been unaffected.

In general this is the result of the action of X-rays upon vitally-stained paramœcia. The quantity of energy necessary to inhibit the activity of unstained paramœcia varies from sixty to eighty milliampere minutes under the conditions of technic given above. When the cultures are stained, as with trypanblau in the concentration mentioned, the amount of energy required to inhibit their activity is reduced to from five to ten milliampere minutes. There is however, considerable variation within certain limits in the susceptibility of the cultures to X-ray energy and also in the rapidity with which they absorb stains.

So far as could be observed upon these specimens the cause of death was due to an assumption of stain by the nucleus. This causative agent appears to be constant both in the stained but not rayed specimens and also in the stained and rayed specimens. It would appear as if the nuclear membrane were normally impervious to the colloid stain until the degree of concentration of the stain in the cytoplasm became great enough to overcome this resistance. One might assume that X-rays alter this permeability thus permitting the stains to diffuse readily into the nucleus.

Solutions of trypanblau, trypan red, neutral red, and of dahlia when exposed to X-ray energy are not fluorescent nor is there a loss of color reaction of solutions from prolonged exposure to the rays. Very careful histological examination under high-power magnification has failed up to the present to detect a change in the color of the absorbed stain after raying, or to give evidence of a redistribution of the stain in the cytoplasm. Moreover, a series of experiments in which normal paramœcia were stained by trypanblau solution which had previously been rayed for periods varying from five to thirty minutes, failed to demonstrate either an injurious effect of the rayed stain upon the organisms or an increased rapidity of absorption by the organisms of that stain. The cause for the increased susceptibility of the organisms to X-ray energy is to be found apparently in some factor which is operative only when X-rays act on the cells in the presence of the stain.

ALTERNATE PHASES IN FOLLICULINA.

E. A. ANDREWS.

As long ago as 1858 it was independently affirmed by Wright (7) and by Claparede and Lachmann (6) that the sedentary marine infusorian once known as Freia, but called now by the more prosaic term, the bottle animalcule, *Folliculina*, could reduce itself to a more simple larva-like form and then swim free, subsequently to make for itself a new dwelling in which to differentiate again into the perfect form.

So great was the difference in structure that the free swimming form was regarded by Daday (3) as a new genus to be placed in a family quite different from the family of Stentors in which the adult sedentary *Folliculina* belongs.

Even as late as 1916 Sahrlage (4) after much study of *Folliculina* denies the reality of any process of alternation of differentiated and dedifferentiated phases in *Folliculina.* He also denies the credibility of the evidence that *Folliculina* is found in fresh waters as well as in the sea.

In both these negations he is shown to be wrong by Penard (5) who studied fresh water Folliculinas and describes the transformations from one phase to the other back and forth.

However, both these authors, overlooked the fact that in 1914 (1, 2) the same alternation of sedentary and free swimming phases early claimed for *Folliculina* in Europe had been found in American waters.

The present paper will serve to reaffirm the reality of this alternation in Folliculina and to emphasize its fundamental nature.

Folliculina being the most specialized of the highly complex Stentors presents in its anatomy an unusually high grade of complexity, while its ability to fabricate a complex dwelling by secretion is the acme of accomplishment among the ciliated protozoa.

Under the conditions elsewhere described (1, 2) *Folliculina* passes readily back and forth from more complex sedentary to more simplified free-swimming phases of form and activity.

In the sedentary phase the animal occupies a house or case from which it may protrude two very long ligulate lobes that make its feeding apparatus so effective. In this phase the animal presents the maximum differentiation and is capable of division, as described in part by Mobius (9) and more in detail by Sahrlage (4). This is the adult or perfect phase. In the free-swimming phase the animal lacks the feeding lobes, the mouth and the special organ for attachment and it is in fact a simplified, retrograded or dedifferentiated form which some have called a larval form. It is, however, the free-swimming form that fabricates the house which it will occupy when it has differentiated into the perfect form, from which state it may again retrograde back into the simplified free-swimming stage and again leave its house.

Briefly, the general structure of these two forms is as follows: The free-swimmer is cylindrical, capable of great extension and contraction with spiral and lateral bendings. It moves by means of about 100 lengthwise lines of cilia. At the posterior end the cilia are absent over an area within which is a complex, problematical organ into which the myonemes converge. At the anterior end there is a very small, nearly circular spiral of membranells which though active seem to have no special use. Where the infundibulum and mouth might be expected there is a small microscopic vestige of no conceivable use. The animal captures no food. The food that may be inside of it was obtained in the previous phase when the feeding lobes and mouth were present. Essentially this free-swimmer is the adult broken loose from its former attachment to the bottom of its house or case and dedifferentiated to the extent of entire resolution of its lobes, pharynx, and hypostome. The only vestige of all its former feeding apparatus being the above-mentioned minute ectosarcal cup and the single limb of a spiral hypostome.

On the other hand the anatomy of the adult sedentary form is more complicated. In addition to the long lines of cilia and pigment underlaid by very highly specialized myonemes, there is at the posterior or foot end a special organ of fixation to the bottom of the house and at the oral or anterior end the remarkable lobes, arms, or flat ribbon-like extensions of the body which have been likened to the ears of a rabbit. These like the rest of the body are exceedingly pliant and almost always in active movement. Where the two arms or lobes arise from the main mass of the body a funnel leads into the interior and ends in a mouth with complex activities. In addition to the longitudinal lines of cilia which run on both the outer and inner faces of each lobe there is a very highly organized band of membranells. This starts near the edge of the funnel ventrally on the right side, runs out along the entire inner face of the right lobe almost to its tip, then returns parallel to itself along the dorsal part of that same lobe to the main body, makes a dorsal course around the funnel to the left side and out along the dorsal edge of the left lobe almost to its tip, then back parallel to itself near the ventral edge of the left lobe to the edge of the funnel down which it goes in a spiral of about 2 turns, ending very near the cytostome. On the left ventral face of the exterior of the body there is a definite region which opens and closes as the functional anus.

While the two phases thus differ remarkably in the high specialization of the adult feeding apparatus as compared with its nearly obliterated condition in the free-swimmer, both phases have essentially the same character of nucleus, namely one long many-lobed moniliform macro-nucleus accompanied by very many minute micro-nuclei. The function of the free-swimmer is not only to transport the animal from one place to another, often for considerable distances, but also to build up the house in which it will live after it has become differentiated into the adult form.

The method of making the house will be described elsewhere but it consists of a series of activities; first, the selection of the site; second, the making of the sac or bottom; third, the making of the spiral tube; and finally, the finishing of a lip around the orifice on top of the tube. After all this is accomplished, the free-swimmer inside the case that it has built, rapidly differentiates into the adult form, capable of stretching out of the case and getting its food. It is this adult form which may later dedifferentiate or retrograde into a free-swimmer and break loose and escape from the house and later construct a new house somewhere else.

The times consumed in the various elements of this alternation of phases vary very greatly, being hastened by high temperatures in general. Often the animals that have been feeding adults during the night pass into the swimming form in the morning, settle down toward the middle of the day and transform into adults again before night so that an entire wave of rise to the adult and recession to the swimming form and rising again to the adult may be about 24 hours. However, in some cases an entire alternation was accomplished in perhaps as little as 6 hours.

The following anecdote relating to a single individual folliculina acting under unusual conditions may serve to illustrate the pertinacity with which these alternating phases are adhered to and to raise a number of questions which it would require experimentation to solve.

August 28, 1914 which was very near the end of the season for the occurrence of *Folliculina* in the Severn River, a number of perfect animals in their houses were placed in a hanging drop over water in a hollow slide for the purpose of seeing how they retrograde into the free-swimming form. One group had 17 or 18 perfect cases, another had 5.

By some accident one of these cases had the tube jammed at the tip so that it was closed off. August 29 at 7:50 A.M., temperature 75° F., the folliculina in this tube which from its color was evidently an old one, not recently formed, was found to be in a late stage of reduction of the arms in the process of becoming a free-swimmer (Fig. 1). It revolved on its foot as normal, with reversals of direction and the arms had been, as it were, melted down to a castellated ridge or membrane while the long complex adoral zone was now but a semicircular band running along the edge of the very obliquely truncated end of the body, opposite to the above elevated ridge. At 9:50 the arms were reduced to nothing and the folliculina was ready to break loose as a free-swimmer with normal spiral adoral band (Fig. 2). This larval stage then broke free from its foot and proceeded to go out of the tube as is normal, but was prevented from emerging by the closure of the tube near its tip. Then began a long series of advances and retreats in which the free-swimmer vainly tried to force itself out of the tube end, but did not succeed in boring its way out with the narrower tip of the body and all possible efforts (Fig. 3). After two hours and a half of this free-swimming life inside of the case, the free-swimmer settled down to the bottom of the case and became attached there by its foot end again, thus settling down as it naturally would have done after the same interval of time had it, as normal, swum free through the water to some distant point of contact and selected a new solid surface to become attached to, far from its old dwelling.

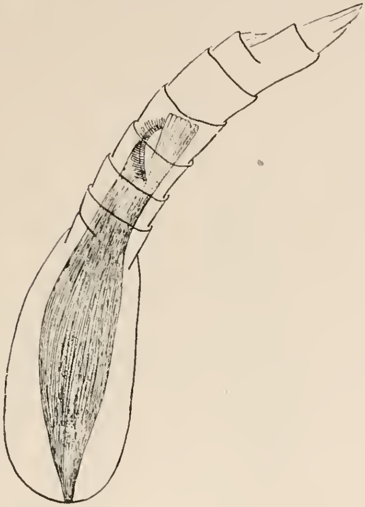

FIG. 1.

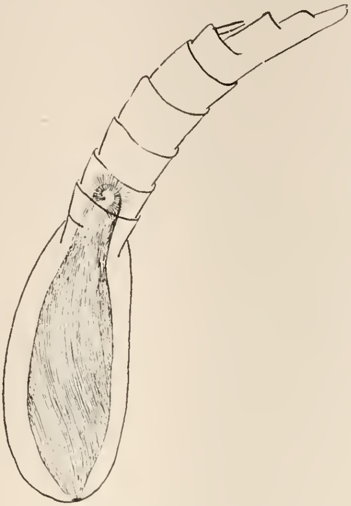

FIG. 2.

FIG. 1. *Folliculina* trapped inside closed case, dedifferentiating its lobes at 7.50 A.M., August 29, 1914.[1]

FIG. 2. Same at 9.50 with lobes gone and free-swimming form completed.

From 12:50 onwards the newly attached larva at the bottom of the case was seen to be reconstructing its lobes (Fig. 4) and this process was completed at 10 P.M. In this process of making lobes (Fig. 4) the usual series of phenomena took place just as is the rule after a free life and formation of a new tube; but here the old tube was used to dwell in and no new tube was formed.

[1] *Explanation of Illustrations.* All sketches outlined with camera lucida, Zeiss, 4, D, that is enlarged about 700 diameters and then reduced to one third in diameter.

All sketches are successive views of one individual *Folliculina.*

In making lobes the old adoral band of membranells sank down into the interior and was apparently entirely melted away during a remarkable loss of organization of all the terminal region of the body accompanied by especial internal circulation of the now very fluid endoplasm, rapid currents passing dorsally from the foot end up to the peristomal end, across that end and down again to the foot ventrally to complete a circuit. The appearances suggest that the usually well organized and rather rigid anterior end of the animal as well as much of the interior rather suddenly breaks down into a fluid state as if a solid were melted, or a gell changed to sol, and then this begins to rotate violently from unknown causes. The animal now is a cone of stiffer ectosarc with ciliated lengthwise bands and subjacent myonemes attached at end, which is tip of cone, while the base of cone is oblique and liquid out to a mere superficial pellicle, apparently (Fig. 4). This oblique soft face has lost all myonemes and cilia as well as the former rigid adoral band of membranells and the pigment patch.

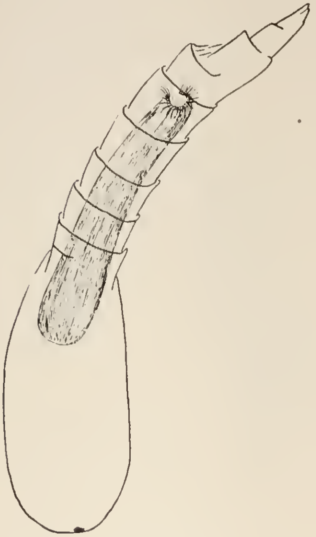

Fig. 3.

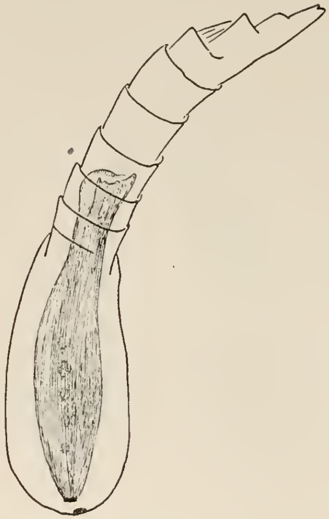

Fig. 4.

Fig. 3. Same at 12.30 swimming in vain attempts to get out.

Fig. 4. Same at 4 P.M. again attached by new base inside case and with new lobes differentiating. A stage like that in Fig. 1 but going toward differentiation and not the reverse.

This oblique cone-base then elongated down one side of the cone like a candle that is gutted down one side, but meanwhile the edges of the soft base rose up more and more as walls like the edges of end of a burning candle. The elevated walls were irregular and fantastic at times, with castellated edges. One side was greater than the other and for long one of the lobes which is the left, was greater than the other. But it is not the elongation of the edges of the cone base that makes the lobes, so much, but the actual splitting down of the base internally into right and left halves. That is, the soft end divides more and more to form a deep groove between the tips of the lobes and so the bases of the lobes are produced more and more down into the interior till finally the two lobes are nothing more or less than the right and left halves of the basal third, or more, of the original cone separated from one another by a deep median space. The new adoral zone that is to follow the edges of the arms arises at an early stage and seems to have no connection with any previous formation but to be differentiated *de novo* from the softened and disorganized terminal and lateral areas. The adoral band starting at one end from within the minor or right arm rudiment, or papilla that is to be its tip, sweeps in a circular arc dorsally and then along the left side central to the rudimental tip of the larger left arm to then pass down the ventral face of the animal where it extends very far toward the apex of the cone. As the animal becomes twisted spirally and has the habit of rotating and of changing the direction of rotation from time to time, it becomes difficult to ascertain how the band is completed, but it is evident that the tip of the band nearest the foot burrows inward into the protoplasm as the future spiral within the peristomal funnel and ultimately acquires a mouth or cytostome at its innermost termination; but in proportion as the two lobes are separated from one another as halves of the animal's body, the two lateral sides of the hypostome curve (Fig. 5) are pulled out as lateral loops that run up and down each arm till the ultimate course of the membranell band is that of two long inverted Us or tuning fork curves connected dorsally by a short dorsal part of the curve, beginning abruptly on the inner face of the base of the right lobe and ending at the opposite base of the left lobe as that inner spiral that runs within the animals peristomal funnel to terminate at the cytostome.

All the complex organizations having been accomplished in usual order, there resulted at 10 P.M., Aug. 29 (Fig. 5) a creature with great powers of extension and with the anterior third of the body split into two very mobile and responsive arms bearing the collecting membranells destined to produce in the water outside the case such powerful currents as rotifers, bryozoa and the close relatives of *Folliculina*, Stentors, make to bring food particles within control of the selective mechanisms near the mouth. As this individual with its newly formed feeding and responsive organs elongated for the first time, as is normal when first reaching out of the tube it had made before possessing such organs, it, in this case of closed up tube, came to a physical resistance that it could not overcome. Very many trials were made in attempts to complete the normal expansion from mouth of tube; sometimes one lobe alone was thrust up into the closed

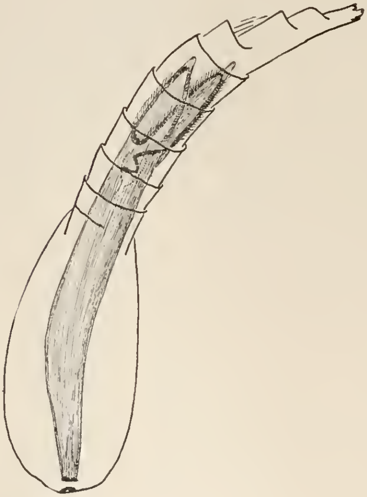

FIG. 5.

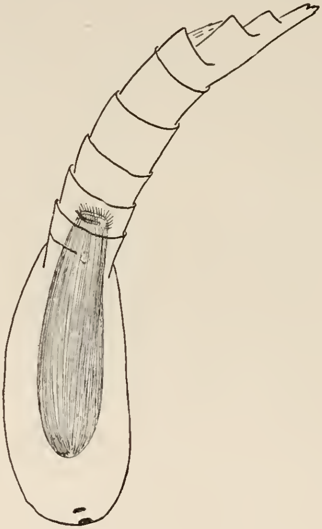

FIG. 6.

FIG. 5. Same at 10 P.M. with fully differentiated lobes, mouth, and feeding apparatus, but with lobes not fully expanded inside closed tube.

FIG. 6. Same August 30, 6.45 A.M., now dedifferentiated into swimming stage again, with two scars of former attachments to base of sac.

tube, the other being bent back alongside of the body like the ear of a lop-eared rabbit; sometimes both lobes were thrust forward. With repeated contractions toward base of tube and repeated expansions and stretchings out, much time was spent in the vain endeavor to complete the expansion which alone would lead to function of the feeding apparatus.

Sometime in the night of August 29 this series of trials must have come to an end, for at 6:45 A.M., August 30, it was found that these useless lobes had been resorbed or dedifferentiated and again the animal was in the form proper to a free-swimming phase (Fig. 6).

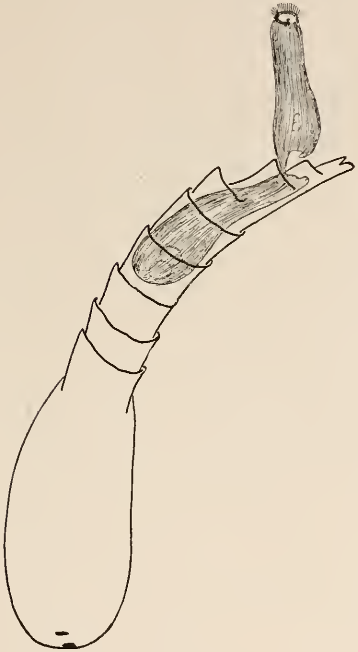

FIG. 7.

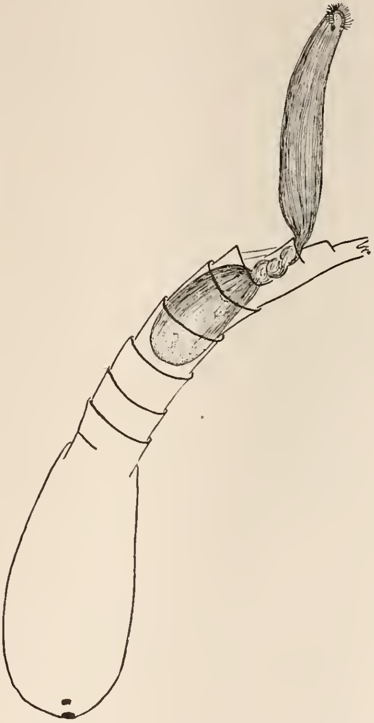

FIG. 8.

FIG. 7. Same at 8.57 when the anterior part had succeeded in forcing its way out of tube, but major part remained inside nearly severed from anterior moity.

FIG. 8. Same at 9.09 showing revolution of anterior part, twisting connection with posterior part inside tube, and healing over of torn base of anterior part.

When this new free-swimming phase broke loose from its foot attachment there were two scars left on base of tube sac, one for each of the two secessive periods of attachment of the animal to the base of the same dwelling.

Upon swimming up to the top of the tube to escape, as is normal, the free swimmer now met with resistance as before but for some reason the tube was not entirely closed as it seems to have been the day before, possibly the force exerted by the pushing of the lobes of the animal the day before had some effect in changing the character of the closure of the tube. At all events the free swimmer was finally able to force its front end out of the tube through a minute orifice on one side back of the tip so that by 8:45 it was seen escaping from the tube (Fig. 7). However, the escape was but incomplete since the hole was minute and the animal did not succeed in propelling its entire length through; on the contrary, more than half its bulk remained behind within the tube (Fig. 7) and the body was constricted to a very narrow waist when passing through the narrow hole, and was, as it were, cut almost into two with a very narrow pedicle connecting the swimming part outside the tube with the enlarged part within the tube. What abnormal conditions here prevailed it was not determined, but one factor in the failure to progress completely out of the tube seems to have been the abnormal state of the foot end. This swelled up into globular form and lost much of its normal organization, receding into a more fluid phase. The contents slowly revolved in flow and the protoplasm seemed more dead than living. Nevertheless the cilia continued to cause the mass to revolve for three and a half hours before dissolution of the ectosarc resulted.

Meantime, for nearly two hours, the anterior part of the animal now outside the tube continued to swim with its numerous rows of cilia and exerted strain upon the part within the tube; this gradually led to the pulling out of a lengthening isthmus or cylindrical waist between the active outside and the passive inside swollen part. The base of the outside part became pinched off nearly to complete severance from the rest. It formed an oblique base of ragged form sending out pseudopodial processes but ever at one corner, continuous with the hind part of the animal by a very slender strand that reached through the hole in the tube to the rest of the animal still imprisoned. Within the tube this slender strand became twisted as the outside part swam in its usual spiral revolving way (Fig. 8). Gradually, this dark green strand was at first twisted, drawn out longer, made an ever lengthening and very slender string connecting the active outside animal with its dying foot (Figs. 8, 9, 10, 11). The animal revolving swung its head end in wide circles while the slender string held its other end firm to the hole in the tube. Moreover, the direction of pull exerted by the combined stroke of the many cilia was changed so that the strain now pulled

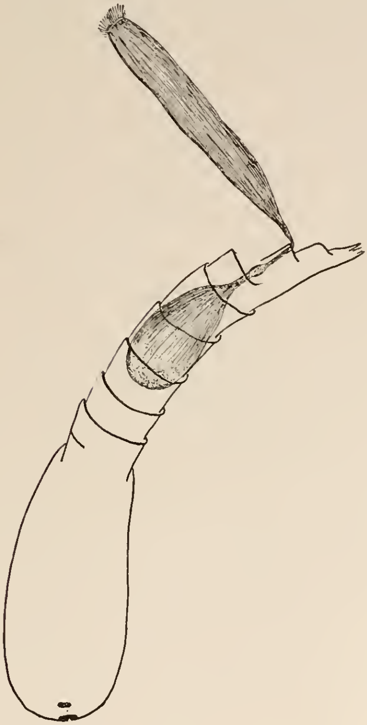

FIG. 9.

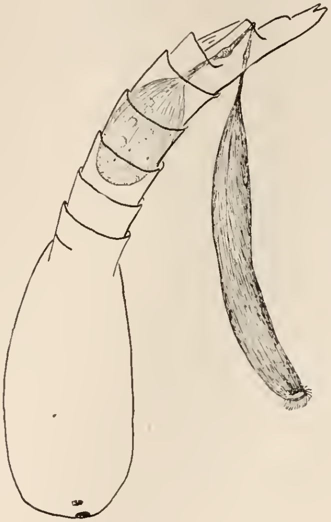

FIG. 10.

FIG. 9. Same at 10.05 when the anterior part swimming powerfully stretches out both its own body and the connecting strand that binds it to posterior region within tube.

FIG. 10. Same at 10.10, showing change of direction of swimming that tends to wind the connecting strand about the tube.

the string out at right angles to the tube (Fig. 9), now parallel to the tube length. Within the tube the string was also pulled out long (Fig. 9) but did not break loose either from the dead swollen foot mass nor from the swimming part. It is to be remarked that the ragged base of the free animal had by 10 A.M., that is, in about an hour's time healed over so that it was no longer ragged, oblique, provided with projections and attached by one corner only to the string, but was smoothly finished as a cone with its central part passing smoothly forth (Fig. 9) as the cylindrical slender string of protoplasm which would not break. With the elongation of this strong filament of firm protoplasm, the free animal had more and more wide excursions bound by this

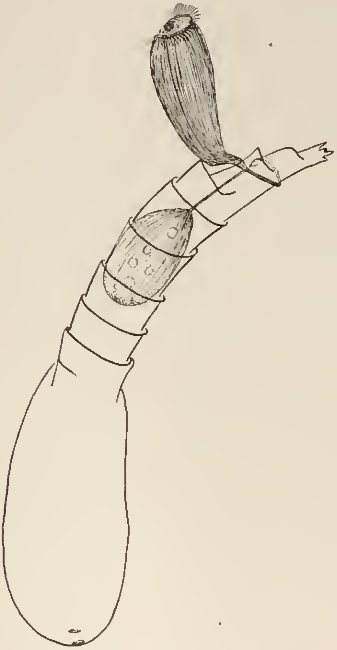

FIG. 11.

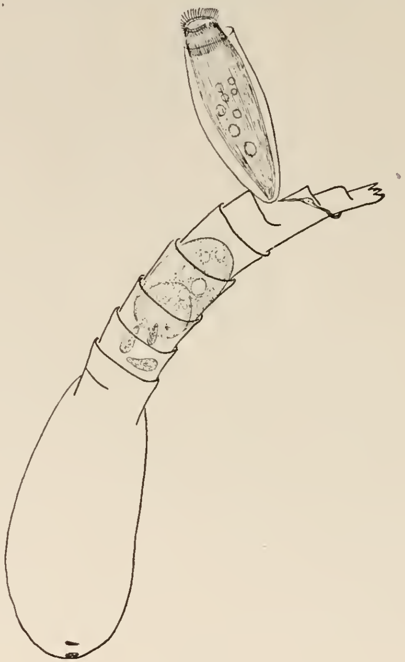

FIG 12.

FIG. 11. Same at 10.40. The strand wound about the tube brings base of swimming part against tube and the swimming form contracts to take on the sedentary phase.

FIG. 12. Same at 11.40. The free-swimming stage having terminated, the anterior part has secreted a sac attached to old tube; while the posterior part is disintegrated and being eaten by scavengers within the tube.

tether, and came to swim entirely around the end of the tube, pulling its cord with it till it became wound up like any larger animal so tied (Figs. 10 and 11). Fig. 10 shows the result of an excursion about the end of the tube in which the string was drawn out very fine and long, but later the animal relaxed and ceased to swim vigorously so that the string shortened and thickened somewhat. Its elasticity pulled the body back toward the tube. The effete mass within the tube still seemed alive on the surface at 9:25 as it had myoneme bands, and cilia that caused it to revolve, but by 10:32 it had come to rest. At 10:40 (Fig. 11) the animal ceased to struggle and now having spent the normal time of a free-swimmer in the swimming activities thus carried on by the anterior part with the limitations of tethering to the imprisoned posterior part, the internal conditions of fixation and tube building had become ripe for expression. The base of the external moity flattened out on the tube surface and acquired attachment to the tube after the manner of a nascent foot, while at the same time breaking off the long-lasting tether or strand that had held it to the posterior moity within the tube. Of the old animal was now left the anterior part which proceeded to make a new dwelling on the outside of the old tube (Fig. 12), the swollen posterior part within the old tube which became more and more a rounded dead mass to be destroyed by protozoan scavengers and the slender connection string or middle of the old body which partly inside and part outside the tube soon was lost to view, as if disintegrated or eaten by scavengers.

The fate of the dead posterior was as follows: A rounded mass (Fig. 12) containing portions of the original macro-nucleus was attacked by protozoa which forced themselves against it and indented it as if it were jelly-like with more firm exterior though the original myonemes and colored bands had disappeared. At 11.53 the scavengers entered into the original foot end of the mass which now had become liquified or disintegrated where attacked. At 1 o'clock the mass was broken up into fine granular matter (Fig. 13) scattered about by the movements of thirteen scavengers and into certain refractive shells, apparently representing lumps of the macro-nucleus of greater firmness eaten out on one side by the scavengers which pushed them about as they ate into them. By 8 o'clock the next morning (Fig. 14), August 31, nothing visible was left to represent the old dead posterior end except a few of the scavengers, six in place of thirteen, and these moved sluggishly, and the following day only dead remnants were left, imprisoned in the old tube base, as greenish detritus. The scavengers seemed to be the ciliated protozoon, *Urotricha*.

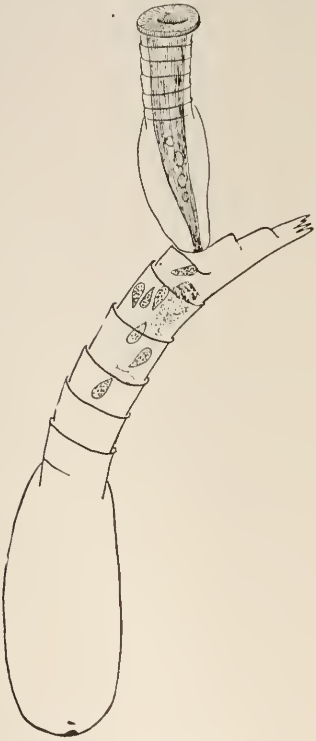

Fig. 13.

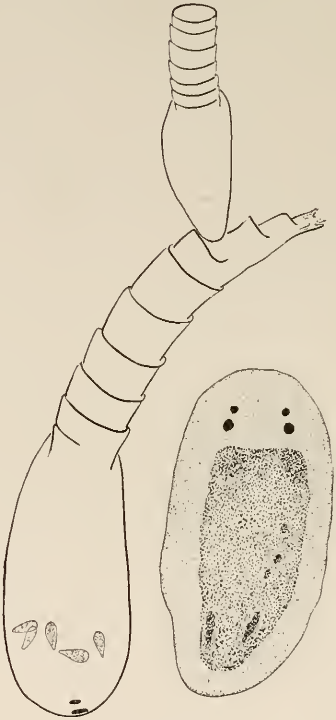

Fig. 14.

Fig. 13. Same at 12.59 after the sedentary form has completed a spiral tube and has assumed the mushroom form preparatory to finishing the secretion with a terminal lip. The old posterior part within tube nearly all eaten up by scavenger protozoans.

Fig. 14. Same August 31, 8 A.M. after a planarian (which is indicated below on right), had sucked out the sedentary folliculina when about to differentiate into the perfect form.

The anterior living part of the animal, however, outside the tube while attached to the tube continued to advance in the normal cycle of rhythmic activities characteristic of an entire animal; that is, it proceeded to make for itself a new dwelling with all the developments of form that underlies that architectural result. To be sure this new dwelling was small as was the bulk of the anterior half animal that made it and it was not as perfect as normal tubes in the open, but yet it was merely lacking in finish and some perfections of proportion and in no wise deficient in fundamental traits nor more unfinished than many dwellings of whole animals when made in conditions of poor food, etc., incident to long stay in a hanging drop.

The process of making the tube was continuously observed for nearly one hour during which time a dozen camera lucida sketches of successive phases were made. In making the dwelling the new animal which had regenerated from the anterior part of old one followed the traditional sequences as below described.

The dwelling or case of Folliculina comprises a sac or attached swollen part and a long tubular continuation which is ornamented by a spiral ridge (Fig. 1). The orifice at the end of the tube is completed normally by a very pretty everted lip, made after the spiral part is finished. In this case of dwelling making, the anterior part animal having become attached, proceeded as if entire, assuming for its attached and adjacent areas the same functions as were normally carried on by the old foot which had degenerated within the tube it was imprisoned in. The first action after becoming fastened by the foot consists in contraction into a form which will be that of the future sac end of the dwelling (Fig. 11) and then in this shortened and swollen form the animal secretes from all its general surface, excepting the anterior face that bears the adoral zone, a material which hardens in the water and forms a complete envelope about the animal partaking of its form and thus molding a sac with end open where the anterior face of the animal projects and does not secrete. This secreted sac lies all in one plane and generally has marked bilateral symmetry, since it is attached along one face which is flattened, but in such examples as the present where standing out rather free into the water the sac (Fig. 12) is not so markedly bilateral.

After the sac is completed it is continued as the tube, but this always forms an angle with the axis of the sac (Figs. 1–14). To produce this abrupt change of angle and to form the spiral tube the animal has the habit of modifying the anterior region to make a sort of temporary head or cylindrical plug which projects out of the sac and acts differently from the region of the animal that remains within the sac. This plug-like head sticking out of the sac like a cork in a bottle turns upward at a large angle to the main axis of the sac, and thus generally at a large angle with the surface of attachment, be it vertically above or below. This angle in the main axis of the animal is maintained during the making of the tube and the tube is made round about the head region, little by little as the head region is extended farther and farther away from the foot by the gradual elongation of the whole animal. The length of the head or secreting region is such as to produce one of the component rings of the tube and it makes first the bottom one, then the next. However, these are not commonly made as mere rings but as parts of a continuous spiral, since, in proportion as the head slowly advances the head revolves and bends laterally, or nutates, secreting successively left, ventral, right, dorsal and so on. There are then three coördinated actions in this making of a spiral tube: first, the slow elongation of the animal, second the contractions of its anterior end to make a mold or form about which secretion is deposited, third the revolution of the head and also what might at first be called a fourth, the localization of secretion so that it is not uniform all round about at any one moment. This latter factor is the least readily made out and involves the appreciation of the fact that the spiral is really bounded by a hollow ridge due to a duplication of the tube wall and this duplication arises from the form and revolution. That is, as one side of the head is more flat it makes the straight walls of tube but as opposite side is bulged out it makes convex ridge and as revolution brings these sides into play successively the bulged out secretion is lined inside by the flat wall. The tube is smooth within but presents a spiral hollow ridge on the outside only.

The animal here considered proceeded normally with this spiral activity till five or six rounds had been finished (Fig. 13) and then made ready to complete the dwelling by the addition of the everted lip. The making of the lip is one of the most specialized associations of form and secretion in the whole gamut of changes that the animal can run through. As indicated in Figs. 12–13 the head that has been held for more than an hour during the secretion of the spiral tube like a short plug or cork in form and position now suddenly changes into a flat terminal disk which projects outward all round about the mouth of the tube like a mushroom cap from the body of the animal as a stalk. In this mushroom, or disk-phase, of the head the adoral zone becomes excentric on the terminal face of the disk and soon disorganizing changes of the central parts initiate the future completion of dedifferentiations that are to be followed by the differentiation of new arms or feeding organs. But first the overhanging rim of the mushroom normally secretes from its lower surface an annular continuation of the tube which lacks any spiral element and terminates the tube as a horizontal shelf, convex on top; that is, an everted rounded collar comparable to the everted lip of many a potter's jar. However, in this particular instance the collar was never completed, since at 1 o'clock, August 30, a small planarian imprisoned in the hanging drop came over the incomplete dwelling of the *Folliculina* and in one minute, thrusting its proboscis into the tube as the *Folliculina* withdrew, with a few gulps, sucked the entire *Folliculina* up into its digestive cavity.

But for this unexpected event the *Folliculina* would probably have soon completed the lip of its dwelling tube and then have differentiated the usual feeding apparatus, with the aid of which it might have accumulated energy and eventually have returned to normal size after successive alternations of form and of activity.

Comparing the alternations of phases of the normal with those in this individual hampered by restrictions, we observe that the normal was nearly attained, except in the function of secreting a house; and in periods of time closely alike in the two cases.

There is here remarkable adherence to the normal rhythm despite changed external conditions. When the feeding form could not expand and feed as normal it retrograded into the swimming mouthless phase and this after the usual time of swimming activity though not exposed to outside environment but still within the same house in which the feeding phase had lived, settled down and differentiated the feeding apparatus again, though it skipped the usual making of a house. This feeding stage not functioning as such again reverted to the swimming phase which partly escaped from confinement but was held tethered for such a long time that the next phase, the settling and house building phase came into expression, with the result that the anterior part of the animal made such a house as is normally made by the entire animal while the posterior part of the animal underwent cytolysis.

This comparison may be put in tabular form:

The Average Folliculina.		The Imprisoned Folliculina.
To use and resolve arms	Many hours	About twelve hours
Only to resolve arms	Several hours	Two hours and more
Free-swimming phase	Two hours and more	Two hours and more (inside house)
To make sac	One half hour	
To make tube	Few hours	
To make lip	Quarter to half hour	All omitted
To make whole house	Four to eight hours	
To differentiate arms, etc.	One and three quarter to four and a half hours	Six hours
Again to use and resolve arms	Many hours	Nine hours or less
Free-swimming phase	Two hours and more	Four hours (partly out of house)
To make sac	One half hour	One hour
To make tube	Few hours	Two hours
To make lip	Quarter to half hour	Seventeen minutes (when stopped undone)

It is then evident that long journeys through the water are not necessary preliminaries to the settling down of the swimmer to secrete and to differentiate. That the use of the feeding apparatus is not necessary preliminary to the dedifferentiation of the complex form into the simple; and that the anterior part of the animal can act like a whole animal. In brief the external conditions are not, for a time at all events, the necessary determining factors in the transformations back and forth from sedentary complex to migratory simple forms, or phases. Some internal factors must be decisive in the alternation of forms.

That time is spent in one phase and again in the next phase suggests a series of internal events that must be accomplished before each phase must give place to the next.

As is often the case in normal specimens the free swimming phases in this imprisoned animal were in the daytime and the sedentary phases in the night-time so that one might suppose the phases to be dependent upon some concomitant of day and night alternations, especially as the animal is deeply pigmented both in its granules and also throughout its plasma, so that it should be affected by light and heat; however, there were many instances where the transitions from phase to phase seemed to be quite independent of alternations of light and darkness. The free swimming forms are very responsive to light and generally positive and this favors their swarming out in the daytime. However the dedifferentiation that precedes swarming may be associated with metabolic changes that have but remote connection with diurnal changes. How far these internal factors may be thought of along the lines suggested by E. J. Lund (8) remains for future experimentation to discover.

As in bursaria so in folliculina the nuclei remain apparently unchanged during the transformations from phase to phase and the transformations are those of the organs of external relation, feeding, muscular and ciliary movements and responsiveness.

Regarding the closure of the tube as presenting problems to be solved by the folliculina, it is difficult to see how an intelligent animal could have solved them more satisfactorily with the implements at hand: and were there a complex nerve system present we might assume activities within it like those of a higher animal, such as would lead to omission of the secretion of a useless dwelling after some tactual appreciation of the existence of one formerly secreted.

Granting some internal sequences that lead to alternate forms despite usual or unusual external conditions the most difficult question is why the secretion of a dwelling was omitted when the animal was held confined within the old dwelling. The omission of this series of activities that normally takes place like a chain of reflexes, leads to the appearance of a remarkable parsimony, as if the presence of the old dwelling was a signal for the neglect to construct a new one.

In this connection it should be pointed out that in several instances it was observed that free-swimmers intruded into dwellings already occupied and nevertheless when the time for transformation arrived, these intruders, one in each tube, secreted a new house within the host's house; thus showing that the presence of a surrounding house need not necessarily inhibit the making of a house within a house. In such instances also there was no intelligent result, since the new house blocked the passage and neither host nor guest was ever able to feed nor to escape from the predicament (except in one instance when a tear in the side of the old tube enabled both animals to protrude their feeding arms).

SUMMARY.

In the protozoan *Folliculina* an individual imprisoned continued to pass from differentiated to dedifferentiated phases, though both were exposed to the same restricted conditions.

Imprisoned in the night of August 28 the feeding adult dedifferentiated into a swimming form which was active within the prison all the forenoon of August 29.

It then passed into the sedentary phase and omitting to construct the usual dwelling, being inside the old one, differentiated again into the perfect form by ten o'clock.

During that night it again dedifferentiated into the swimming form, still within the old dwelling to the bottom of which it had twice fastened itself.

At 8:40 in the morning of August 30 this swimming form had partly emerged through a rent in the dwelling.

This protruding anterior part of the animal swam violently, held tethered to the dying posterior half left within the dwelling.

At ten forty the free-swimming part settled down on the outside of the dwelling and carried out the normal series of acts in fabricating a new dwelling attached to the old one and almost completed at 12:40.

But at one P.M. the animal was destroyed and rhythmic alternation thus ended.

It is inferred that these alternations of form with differentiation and dedifferentiation are not directly dependent upon such external conditions as exposure to long stretches of water, feeding,

nor probably, upon direct concomitants of day and night successions.

The nature of the internal factors responsible for this apparent striving to complete the normal alternations of phase, and the causes underlying the omission of the usual making of a dwelling, which would in this case be redundant, remain to be found out. Were there a complex nervous system we might refer much of these activities to it.

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BIOLOGICAL BULLETIN

ENTOMOSTRACA AND LIFE ZONES.

A Study of Distribution in the Colorado Rockies.

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I. The Region Covered.

In a former paper ('17) I described the altitudinal distribution of the 71 species of Entomostraca known to occur within the limits of the state of Colorado. Fifty-five of these species are from my own collections made in a definite attempt to gather a reasonable amount of information about the nature of the entomostracan fauna at different elevations in this part of the Rocky Mountains. The material included 280 vials of plankton from 124 lakes and ponds at elevations ranging from 4,100 to 12,188 feet above sea level. By far the greater number of these collections are from an area covering adjacent parts of the counties of Boulder, Jefferson, Gilpin, Clear Creek, and Grand, and includes, within a distance of 25 miles, portions of all the life zones from the Upper Sonoran to the Arctic-Alpine. A full description of the topography and climate of this region is included in my former paper. In addition to my own collections from this region, together with a few from other parts of the plains of the state, I have made use of all available records of species collected by others within the state.

The state of Colorado affords an area for study which is not wholly an arbitrary or unnatural one. Its higher portions include the greatest elevations of the Rocky Mountains, with their southern extension of climatic and biotic characters from the north, and its lower portions are typical of the arid and hot conditions of the southwestern portion of the United States, while its position astride the Continental Divide makes it the meeting place of eastern and western species. Its biota is accordingly composed of a mixture of northern and southern, of eastern and western forms. Reports on plants, birds, and mammals testify to the richness and variety of life here where so diverse conditions are condensed within a restricted area.

In my former paper I referred the Entomostraca of my list to

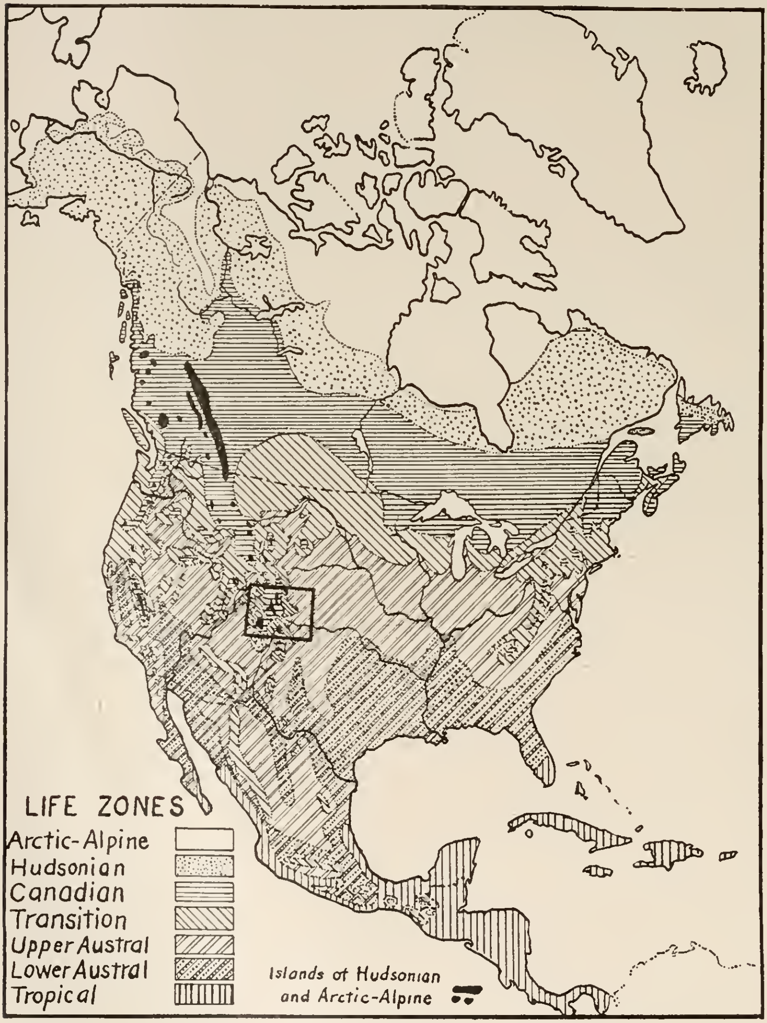
LIFE ZONES
Arctic-Alpine
Hudsonian
Canadian
Transition
Upper Austral
Lower Austral
Tropical
Islands of Hudsonian and Arctic-Alpine

FIG. 1. Life-zone map of North America with outline of state of Colorado drawn in. After Merrian, etc., from A. O. U. Check-list of North American Birds, 3d edition (revised), 1910.

the modification of Schimper's zones adopted by Ramaley in his studies of the vegetation of the area. It seems desirable, however, to tie this fauna to the larger life zones which apply throughout the continent as a whole and which have been mapped out by Merriam and are now made use of by a considerable number of those interested in problems of distribution. Figure 1 shows how these zones are distributed over the continent. Of special interest are the southward extensions and islands of the northern zones over the higher areas, as in the Appalachians, Rockies, and Sierras. The outline of Colorado, indicated by the black rectangle, shows the position of the area under consideration with reference to one of these southward extensions. Cary ('11) publishes a more detailed life-zone map of the state, a small portion of which is reproduced in Fig. 2. This includes the area from which most of my records come and shows that within a few miles are met Upper Sonoran, Transition, Canadian, Hudsonian, and Arctic-Alpine Zones.

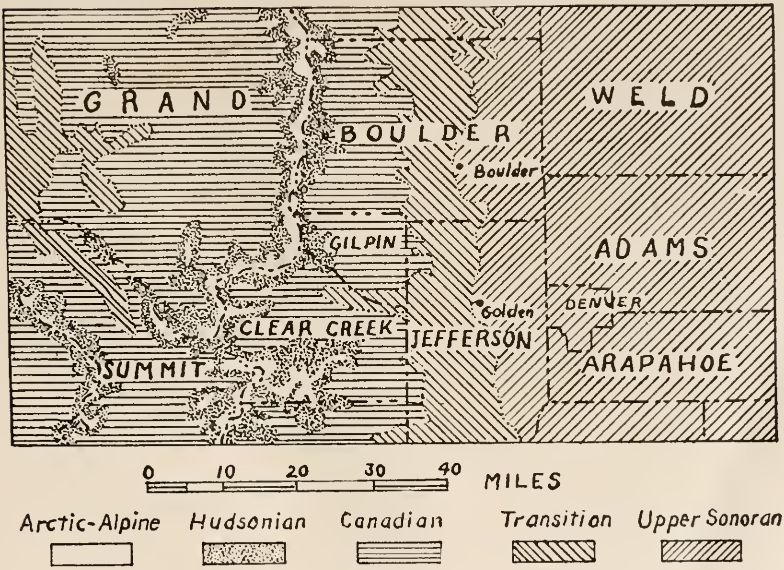
GRAND
BOULDER
Boulder
WELD
GILPIN
ADAMS
Golden
DENVER
CLEAR CREEK
JEFFERSON
SUMMIT
ARAPAHOE
0 10 20 30 40 MILES
Arctic-Alpine
Hudsonian
Canadian
Transition
Upper Sonoran

FIG. 2. Life-zone map of the portion of Colorado where most of the writer's collections were made. Shows close approach up Upper Sonoran and Arctic-Alpine Zones and narrowness of other zones in this region. After Cary.

The approximate altitudinal limits and outstanding floral characters of these zones in this part of the state as given by Cary and verified by my own observations, are as follows:

1. *Upper Sonoran* (arid, western portion of Upper Austral): Western edge of the Great Plains as far as the base of the foothills (about 5,300 feet). Grassland with deciduous trees along watercourses only.

2. *Transition:* The foothill region up to about 8,000 feet: Characterized by an open growth of rock pine (*Pinus scopulorum*).

3. *Canadian:* Includes the intermediate mountain elevations up to about 10,500 feet. Lower portion characterized by a dense growth of lodge-pole pine (*Pinus murrayana*) which gives place at about 10,000 feet to a close forest of Engelmann spruce (*Picea engelmanni*) and balsam fir (*Abies lasiocarpa*).

4. *Hudsonian:* A rather narrow strip characterized by a dwarfing of the spruces and firs of the Canadian Zone and bounded above by timberline, a sharply defined but jagged division line occurring roughly at about 11,000 feet.

5. *Arctic-Alpine:* An area without trees or woody plants except some scrub willows. Huge snowdrifts last far into the summer or do not entirely melt. Summer temperatures are accordingly low and the season is short.

II. The Species of Entomostraca.

Table I. gives the list of species recorded for this area, and indicates for each the zones within which it has been collected. Of course it must be recognized that to make these data of final importance, very much more extensive collections are needed. It is apparent, however, that even the incomplete records here presented are sufficient to bring out facts of interest. The records for the more abundant species are sufficient to give wholly dependable results while the scattered records of the rarer ones are accurate as far as they go, but leave deficiencies and blanks to be filled by subsequent collections.

I have also included in Table I. the zones inhabited by each of these species throughout the Continent as a whole. These data are in many cases incomplete and in others uncertain because I have not had access to literature where I might find detailed ac-

TABLE I.

SHOWS ZONAL DISTRIBUTION AND GENERAL RANGE OF EACH SPECIES RECORDED FROM COLORADO.

Zones occupied in Colorado, as indicated by local collections, are designated in the upper line: LA, UA, T, etc. The probable distribution among the life zones throughout the continent of North America is indicated in the lower line: la, ua, t, etc. The Tropical Zone is omitted because of difficulty of determining with certainty which species enter it. A brief statement of general range is given in the last column. Records in parenthesis are for species not in my collections.

Species.	Number of Lakes	Zones.						Range.
		Lower Austral.	Upper Austral.	Transition.	Canadian.	Hudsonian.	Arctic-Alpine.	
Branchinecta coloradensis								
Packard.............	7		UA				AA	Colorado .
Diaptomus shoshone Forbes	39				C	H	AA	Colo., Yellowstone.
D. arapahoensis Dodds...	4						AA	Colo.
D. coloradensis Marsh....	29			T	C	H	AA	Colo.
D. lintoni Forbes... ...	2				C			Colo., Yellowstone.
D. judayi Marsh........					(C)			Colo.
D. nudus Marsh........	7			T	C	H		Colo.
D. leptopus var. *piscinae*								
Forbes...............	27			T	C	H		Colo., Mont., Al-
				t	c	h?		berta, Manitoba.
Macrothrix montana Birge						(H)		Rockies and Sierras.
						h		
Limnetis gouldii Baird....	2			T	C			Ill., New England,
			ua	t	c			Quebec.
Latona setifera (O. F. M.)	1				C			Northern U. S. and
				t	c			Europe (rare).
Holopedium gibberum Zad-					C	H		Yellowstone,Sierras
dach........	11			t	c	h	aa	North U. S. and
								Europe, New-
								foundland, Green-
								land, Iceland,
								Alps.
Eurycerus lamellatus				T	C	H		Yellowstone, Sier-
(O.F.M.)............	10			t	c	h	aa	ras, North U. S.
								and Europe, Ice-
								land.
Acroperus harpae Baird ..	12			T	C	H		Yellowstone, Sier-
				t	c	h		ras, North U. S.
								and Europe.
Depanothrix dentata	2				C			Me., Mich., Wis.,
(Euren)..............				t	c			Europe.
Camptocercus rectirostris	2				C			Sierras, U. S.,
Schoedler......				?	c	?		Europe.
Pleuroxus procurvatus	5			T	C			Northern U. S.
Birge....				t	c			
Alonella exigua (Lillje-	1				C			Me., Mich., Wis.,
borg).......... ...				t	c			Europe.

Table I.—*Continued.*

Species.	Number of Lakes.	Zones.						Range.
		Lower Austral.	Upper Austral.	Transition.	Canadian.	Hudsonian.	Arctic-Alpine.	
A. excisa (Fischer)	2			T				U. S., Europe, Greenland.
			ua	t	c	h	?	
Canthocamptus staphylinoides Pearse	4				C	H	AA	Nebraska.
			ua					
Streblocerus serricaudatus (Fischer)	1				C			La. to Wis., Norway, Sweden, Europe in general.
		la	ua	t	c	?		
Macrothrix hirsuticornis N. & B.	11		UA	T	C	H	AA	Maine to Colo. Spitzbergen to north Africa.
		la	ua	t	c	h	aa	
Alona affinis (Leydig)	15		UA	T	C	H	AA	America and Europe from north to south.
		la	ua	t	c	h	aa	
Alona guttata Sars	4		UA	T	C			Wide spread in America and Europe.
		?	ua	t	c	?		
A. rectangula Sars	22			T	C	H	AA	Wide spread in America and Europe, Pribilof Ids.
			ua	t	c	h	aa	
Daphnia longispina O.F.M.	48			T	C	H		Cosmopolitan with many varieties.
		?	ua	t	c	h	?	
Daphnia pulex DeGeer	48		UA	T	C	H	AA	Cosmopolitan with many varieties.
		la	ua	t	c	h	aa	
D. hyalina Leydig	1		UA	T	C			Wide spread in Old and New Worlds.
		?	ua	t	c	h	?	
D. psittacea Baird	6		UA					U. S., Greenland to Algiers.
		la	ua	t	c	h	aa	
Ceriodaphnia reticulata Jurine	22		UA	T	C	H	AA	Cosmopolitan.
		la	ua	t	c	h	aa	
C. pulchella Sars					(C)			Cosmopolitan.
		la	ua	t	c	h	?	
Chydorus sphaericus (O.F.M.)	71		UA	T	T	H	AA	Cosmopolitan.
		la	ua	t	c	h	aa	
Cyclops albidus Jurine	16		UA	T	C	H	AA	Cosmopolitan.
		la	ua	t	c	h	aa	
		la	ua	t	c	h	aa	
C. bicuspidatus Claus	38		UA	T	C	H	AA	Northern portions of America and Europe.
			ua	t	c	h	aa	
C. serrulatus Fischer	30		UA	T	C	H	AA	Cosmopolitan.
		la	ua	t	c	h	aa	
C. viridis Jurine	45		UA	T	C	H	AA	Cosmopolitan.
		la	ua	t	c	h	aa	
Graptoleberis testudinaria (Fischer)	7		UA	T	C	H		U. S. and Europe. Greenland.
		la	ua	t	c	h	aa	
Simocephalus serrulatus (Koch)						(H)		Cosmopolitan.
		la	a	t	c	h	?	
S. vetulus (O.F.M.)	26		UA	T	C	H		Cosmopolitan.
		la	ua	t	c	h	?	
Scapholeberis mucronata (O.F.M.)	14		UA	T	C			Yellowstone, Sierras, Greenland. Arctic to tropics.
		la	ua	t	c	h	aa	

TABLE I.—*Continued.*

Species.	Number of Lakes	Zones.						Range.
		Lower Austral.	Upper Austral.	Transition.	Canadian.	Hudsonian.	Arctic-Alpine.	
Bosmina longirostris (O.F.M.)	3	la	UA ua	t	c	h	?	Yellowstone, Sierras. America and Europe from north to south.
Canthocamptus minutis Claus	1	la	(UA) ua	t	C c	h	?	Wide spread in America and Europe.
Dunhevedia crassa King	3	la	ua	T t	C c			La. to New England and Wis. Europe, Australia.
Streptocephalus coloradensis Dodds	4		UA	T	T			Colorado.
Lepidurus bilobatus Packard				(T)				Colorado.
Estheria mexicana Claus		la	(UA) ua	t	c			Lake Winnipeg to Mexico; Ohio to Rocky Mts.
Diaptomus signicauda Lilljeborg				(T) t	c			Colo., Sierras.
D. siciles Forbes			(UA) ua	t	c			Neb., Wis., Yellowstone, Muskoka Lakes, Great Lakes.
Pleuroxus adunctus Jurine	1	la	UA ua	t	c	?		Colo., Calif. Northern Europe to Algiers.
P. denticulatus Birge	3	la	UA ua	t				All parts of U. S.
Leydigea quadrangularis (Leydig)	1	?	UA ua	?				U. S. and Europe.
Kurzia latissima (Kurz)		?	(UA) ua	?				U. S. and Europe.
Moina brachiata (Jurine)	7	la	UA ua	t				Wis. and southward. Europe to Egypt.
Cyclops ater Herrick		la	(UA) ua	t				Minn. and Mich. to Alabama.
M. affinis Birge		la	(UA) ua	t				Wis. to La.
Diaptomus pallidus Herrick	1	la	UA ua	t				Wis. to La.
D. siciloides Lilljeborg	2	la	UA ua	t				Wis. to Texas and Calif. (L. Tulare).
D. claviceps Schacht	2		UA ua					Colo., Iowa, Neb.
D. albuquerquensis Herrick	1	la	UA ua					Colo., N. M., Mexico City.
Marshia albuquerquensis Herrick	1	?	UA ?					Colo. and (N. Mex. ?)
Apus lucasanus Packard	2	la	UA ua					Colo., Kan., Lower Calif.
A. aequalis Packard	1	la	UA ua					Colo., Kan., Texas, Mexico, Lower Calif.

TABLE I.—*Concluded.*

Species.	Number of Lakes	Zones.						Range.
		Lower Austral.	Upper Austral.	Transition.	Canadian.	Hudsonian.	Arctic-Alpine.	
A. mewberryi Packard..			(UA)					Colo., Utah.
A. longicaudatus Leconte		?	(UA) ua	?				Colo., Neb., Texas, Montana.
Eulimnidia texana Packard..........		?	(UA) ua					Colo., Neb., Kan., Texas.
Estheria morsei Packard	2		UA ua					S. Dak. to Colo.
E. compleximanus Packard	1	?	UA ua					Colo., Kan., Lower Calif.
Streptocephalus texanus Packard............	2	?	UA ua					Colo., Neb. to Texas.
Thamnocephalus platyurus Packard........	1		UA ua					Colo., Kansas.
Branchinecta packardi Pearse.............	2		UA					Colo.
B. lindahli Packard....			UA ua	t?				Colo., Kan., Neb., Wyoming.

counts of localities where each species has been collected. Many of the records are indefinite or very general and do not enable one to judge just what zone is included. The tabulation, in spite of this, contains much of truth and I have attempted to confine its errors to those of omission. The Tropical Zone has been omitted, chiefly because I have been unable to determine certainly which species in my list extend into it. It is likewise difficult to determine which species range into the Arctic-Alpine.

The agreement between the local and general zonal range is striking, and in many cases, complete. The only departures from this agreement are in the cases of certain species for which the local records do not cover as many zones as the general. These species are mostly those which are not abundant and have been met with infrequently in the Colorado collections, such as certain species which are never present in more than very small numbers and may easily be missed in making collections, or others, which though found occasionally in abundance, are met with in only a small proportion of the lakes in the zones which they inhabit. More extensive collections would doubtless extend the records of many of these species to cover other zones and so bring the local records into still closer agreement with the general.

III. The Fauna of Each Zone.

Table II., summarizing the fauna of the different zones, shows the following points of interest. For the species collected in Colorado there is a decrease in number from the Upper Sonoran upward, that is, a thinning out of population as the more extreme conditions of climate are met. An apparent exception is the Transition Zone where the number is less than in the Canadian. This is no doubt due to the fact that in the region assigned to this zone there are very few lakes and the number of collections is accordingly much less than from any other zone. If we add to the number of species actually collected from each zone in Colorado those which from their general distribution are almost certainly to be expected there, the deficiency of the Transition Zone is made up and the general decrease with each successive zone prevails.

Table II.

Summary of Records Given in Table I.

	Upper Austral.	Transition.	Canadian.	Hudsonian.	Arctic-Alpine.
Number of species collected in each zone in Colorado........	44	28	35	23	15
Number of species in the Colorado list to be expected in each zone as determined by comparing local and general records .	52	50	42	34	28
Total number of species collected in Colorado in all zones....	71				

The Upper Sonoran Zone.

In the Upper Sonoran Zone of this region there occur two kinds of ponds or small lakes: transient pools which follow rains, and ponds and reservoirs filled with water from irrigating ditches. These latter are of recent origin, dating back only to the introduction of agriculture into this region. The fact that they are richly populated speaks well for the effectiveness of the methods of dispersal of plankton Crustacea, but the question may well be raised whether dispersal has kept pace with the increase in number of such bodies of water and whether additions to their fauna may not be expected in future years.

Of the 44 species that have been recorded from this zone in Colorado, 28 have not been found in any of the higher zones, though nine or more of them occur in the Transition zone of our northern states and may be expected to extend into the local portion of this zone. This leaves 17 species which apparently do not go above the Upper Sonoran, to which must be added some others which have their greatest abundance here rather than in the Transition into which they extend. The remaining species of the zone, roughly half of its population, range upward into other zones, 9 of them as far as the Arctic-Alpine. Thus we recognize among the species of this zone two groups: the one including euthermic species which range upward across temperature lines into the higher zones, the other composed of stenothermic forms, intolerant of great temperature differences and accordingly confined pretty strictly to the zone. The euthermic species, while they form at least half the fauna of the zone, are less likely to receive consideration on account of zonal distribution because they do not form its distinctive part. To this group belong nearly all the species of the family Daphnidæ, many of the Chydoridæ and four of the five species of *Cyclops,* together with a few from other groups.

Conspicuous in the stenothermic component of this zone are two groups: (1) four species of *Diaptomus* and (2) eleven species of phyllopods (Anostraca, Notostraca, and Conchostraca). These two groups are of interest because, not only in this region, but in general they are represented by stenothermic species. *Diaptomus* forms an important part of the entomostracan fauna everywhere and includes many species, nearly all of which are very narrowly stenothermic and have restricted geographic ranges. The phyllopod group is of special interest here, for, though represented in the fauna of all parts of the country, and of the world, forms a more important part here than in most regions. The species of this group are especially abundant in the Sonoran portion of the Austral Zone, where they flourish in the transient pools of this arid region. It will be seen by reference to the last column of Table I., that these species are all of restricted range and confined to the states which include portions of the Sonoran zone. The species of phyllopods, like those of *Diaptomus,* are nearly all stenothermic. The phyllopods belong in greater abundance to the warmer zones, while *Diaptomus* is especially plentiful in the colder ones.

When we search for distinctive faunal characters of the higher zones we are confronted with a problem somewhat different than in the Sonoran. In no other zone is there even a single species confined to it and at the same time abundant enough to be used as a zone indicator. In certain respects the zones of the mountain region, Transition to Arctic-Alpine, form a unit group, which, as a whole, may be contrasted with the Sonoran. There are 23 species which have been collected exclusively in the mountains, many of them in considerable abundance, which clearly belong to the colder zones. Yet when we attempt to apportion them among the zones it is found that most of them range through two or more zones. There are, however, certain pretty definite faunal characters of each zone, a description of which follows.

The Transition Zone.

In the foothill region of the mountains there are but few lakes or ponds and accordingly few collections have been made and a small number of species recorded from this zone. This leaves some doubt as to the nature of the transition between the fauna of the plains and that of the mountains. We cannot determine whether this is truly a transition zone or if it is one having special and striking characters of its own, though the former view seems more probable. It is further probable that the fauna of this zone partakes more of the nature of the higher than of the lower zones. While there are 23 species which make the Transition Zone their lower limit, there is not one which finds its upper limit in this zone, though from the records of general range, there are some which may be expected to do so when more extensive collections have been made here. Twenty-eight species have been recorded in this zone, but a total of 50 might be expected because certain others of the Colorado list are present in the Transition of our northern states.

The Canadian Zone.

In the belt assigned to this zone there are many small lakes, nearly all of morainal origin, mostly shallow, often without outlet, and commonly surrounded by forest. Their fauna is rich in individuals and species. There have been collected in the lakes of this zone 37 species, and about 8 others might be expected. This zone is not one of importance as a limit in either direction, for no such significance can be assigned to the five species from the Upper Sonoran which make this their upper limit or to the three which find their lower limit here. Its fauna does, however, include a number of species in considerable abundance which are common forms in the continental Canadian and other northern zones. Important among these are *Limnetis gouldii, Latona setifera, Holopedium gibberum, Eurycerus lamellatus, Acroperus harpæ,* and *Pleuroxus procurvatus.* Six species of *Diaptomus* common in this zone, *D. shoshone, D. coloradensis, D. lintoni, D. nudus, D. judayi,* and *D. leptopus* var. *piscinæ* are confined more or less closely and locally to the higher parts of the Rocky Mountains, where they range through more than one zone.

Of special interest in the Canadian Zone of this region is the great abundance and very frequent association together of *Daphnia longispina* (subject to great local variation) and *Diaptomus leptopus* var. *piscinæ,* which throughout this zone form the dominant species, with *Diaptomus coloradensis* as the form of next importance. *D. longispina,* though a euthermic species and found throughout the world in many varieties, has not been found in Colorado below the Transition and has by far its greatest abundance in the Canadian. *D. leptopus* var. *piscinæ* seems to be confined to the Transition, Canadian, and Hudsonian of the Rocky Mountain region, while *D. coloradensis* is a local species and has not been reported outside the mountain region of the state for which it is named. The list of euthermic species is about the same as in the Upper Sonoran.

The Hudsonian Zone.

This is a narrow zone, both in vertical and in geographic extent, and has only slight significance. Climatic conditions are more severe than in the Canadian; more snow falls and it lies longer; the summer is shorter and the lakes are colder. In these respects it approaches the Arctic-Alpine. Its lakes are of two sorts: the one of the same type as those common in the Canadian, the other more like those of the Arctic-Alpine—lakes on the direct courses of the creeks like those higher up but differing from them in that they are surrounded by timber and have considerable silt on their bottoms. Lakes of the former type in this zone have a fauna of much the same nature as in the Canadian, while those of the latter kind have their affinities, though less decidedly so, with the Arctic-Alpine. A few species make this zone their upper limit and it seems probable that it may have some importance in this respect. In the main, it seems to be a transition zone, one having no important distinctive characters and it might well be included within the Canadian.

The Arctic-Alpine Zone.

This zone is an interesting one partly because of a type not familiar to us, and partly because its fauna is distinctive and striking. The lakes of this zone lie in the deep cirques at the heads of the streams and on the stream courses not far below, and are all just at or above timberline. Most of them are deep and clean and are bordered by steep, rocky slopes. Ice remains till June or July or even later, and the snowdrifts on the surrounding walls furnish cold water throughout the greater part of the summer so that the highest temperatures reached range from 45° to 55° F. Shallow pools of this region have about the same type of fauna and must clearly be classed along with the lakes of this zone.

Fifteen species have been collected here, nine of which are widely euthermic and range through all the zones, not only to the Upper Sonoran of Colorado, but into the Lower Sonoran and Austral, and in some cases into the Tropical. Nearly all of the more abundant species of the zone belong to this group, as *Daphnia pulex, Chydorus sphaericus, Alona rectangula,* and four species of *Cyclops.* Species of more restricted zonal range are, however, important and abundant, though none of great importance is confined to the zone. Most of the northern species listed as of importance in the Canadian fail to reach this zone, but find their highest range in the Hudsonian, though it appears from records of their presence in Greenland and other high northern regions that they might be expected here. An abundance of collections in this region has not, however, found them in the Arctic-Alpine of this region, and we must omit them from its list.

The two most conspicuous and abundant species are *Diaptomus* *shoshone* (33 lakes out of 43) and *Daphnia pulex* (27 lakes). One or both of these species were found in 39 of 43 lakes in this zone. *Diaptomus shoshone* is a large and brilliantly colored species, was described by Forbes from the Yellowstone, and has been reported only from that region and high portions of the Rockies in Colorado. It ranges into lakes far within the Canadian Zone, but has by far its greatest abundance in the lakes and pools above timberline. *Daphnia pulex* is a cosmopolitan and euthermic form but is much more abundant in these high lakes than in any other part of the mountains, in fact is nearly wanting from the Hudsonian and Canadian, where it is replaced by *Daphnia longispina* just as *Diaptomus shoshone* is replaced by *D. leptopus* var. *piscinæ*. The variety of *Daphnia pulex* found in these lakes is unusually large, and though subject to considerable local variation, comes close to the form described from the Yellowstone as *D. clathatra* by Forbes, where, too, it is associated with *D. shoshone*. One of the striking and outstanding features about zonal distribution in this region is this definite and constant association of a species of *Daphnia* with one of *Diaptomus*. The two combinations are striking and constant, one belongs definitely to the Canadian, the other to the Arctic-Alpine, and the reverse combination is seldom met. Neither member of the Canadian pair has been collected from lakes in the Arctic-Alpine, and though both members of the alpine pair do sometimes invade the Canadian, they usually go together and are found in lakes where the other pair is wanting. *Diaptomus coloradensis* is frequently found in the Arctic-Alpine but is of secondary importance just as in the Canadian.

Another species of importance in this zone is the fairy shrimp, *Branchinecta coloradensis* which is commonly found in the pools, though never in the lakes, along with *Daphnia pulex* and *Diaptomus shoshone*. This species was described by Packard from an elevation of 12,000 feet on Gray's Peak, has been repeatedly found in these high pools, and has come to stand as a type of a distinctly alpine species of narrow range (Shantz, '05). Only once have I collected it below the Arctic-Alpine, this at 9,575 feet, well within the Canadian Zone, and there is was associated with the same species of *Daphnia* and *Diaptomus* as in the higher regions. I have one record, however, from a collection dated May 30, 1912, sent me by Professor Max M. Ellis from St. Vrain, Colorado, at an elevation of about 5,100 feet and well within the Upper Sonoran. It seems improbable that there can be any mistake about the label of this collection, and I have repeatedly looked at the material in an attempt to detect differences which would relieve me of the necessity of referring it to this species. This record places it at once in the Upper Sonoran and calls for a revision of our notions of its distribution.

IV. Summary and Discussion.

The zonal distribution, local and general, of the 71 species included in this report affords good material for the study of certain characteristics of euthermic and stenothermic species belonging to closely related families and genera. It is at once apparent that the total entomostracan population of this region is readily divisible into these two components. The euthermic species not only range through several zones in their local and general distribution, but a large proportion of them are essentially cosmopolitan, being reported from all parts of the world where collections have been made. The stenothermic species are divisible into two groups, one belonging to the colder, the other to the warmer zones. The stenothermic species of the colder zones may in turn be resolved into two groups, one of which includes species that range over a considerable area from Colorado northward into the corresponding zones of our northern states, Canada, and even into Greenland, Iceland, and northern Europe, while the other includes species with very narrow ranges, confined to the higher parts of the Rocky mountains. The stenothermic species of the warmer lakes (the Upper Sonoran of Colorado) may likewise be divided into two groups, one, of species ranging over the Sonoran, and through the Austral in general, and into the Tropical Zone, the other, of those with a narrow range in the Upper Sonoran of a few states.

It is further evident from a scrutiny of the facts presented in Table I., where the species are arranged on the basis of temperature toleration, that such a grouping bears a certain relation to taxonomic groups, inasmuch as some of these groups are largely stenothermic and others euthermic. The outstanding features as seen in the Colorado list are as follows:

The 16 species of phyllopods in the list are, with two exceptions, narrowly stenothermic and all but one of the stenothermic species belong to the warm-climate fauna. It is moreover true that nearly all of the known species of the group are stenothermic and have narrow geographic ranges. Among the Cladocera; the Family Daphnidæ is represented by 11 species which are nearly all euthermic in a broad sense, are found at all elevations and in all zones, and are decidedly cosmopolitan, while the Chydoridæ has 16 species about equally divided between stenothermic and euthermic types. The Copepods include two sharply contrasted genera, *Cyclops* and *Diaptomus,* both of which form important components of the entomostracan fauna in all parts of the world. *Diaptomus* has 13 species in the Colorado list, not one of which is euthermic in any broad sense, and all of which are confined within areas of very limited extent. *Cyclops,* on the other hand, with only 5 species, is fully as important. Three of these are broadly cosmopolitan and euthermic, a fourth (*C. bicuspidatus*) is nearly so, being absent only from the warmer waters, and the fifth (*C. ater*), confined to the United States and not found north of the Transition Zone, has as great a range as the widest spread species of *Diaptomus.*

I believe the collections of Entomostraca described in this paper are the most extensive that have been made in this country from a region where high mountains cause such a decided narrowing of life zones. So far as I know, this is also the first attempt to define the entomostracan fauna of the various zones of such an area and to definitely place them with reference to Merrian's Life Zones. The data from Colorado lend themselves well to such an anaysis. The zonation is definite, even though the present collections do not enable us to discriminate between all of the colder zones. It is equally difficult, on the basis of our present knowledge, to sharply differentiate these same zones in their continental extent in Canada and northern United States.

Yet it seems well established by these data that the entomostracan population of the various life zones as they occur in the mountains of Colorado are but attenuated southern extensions and isolated fragments of the broader life zones, from Transition to Arctic-Alpine, which extend from east to west across the continent. Though these zones have been established and defined on the basis of organisms other than entomostraca, yet the agreement is striking and real and extends even to common species inhabiting the same zones in localities hundreds of miles apart and separated by many degrees of latitude.

In this fact we are brought again to a recognition of the truth that change in elevation may, within a very few miles, produce climatic and biotic differences which are brought about by latitude, only after hundreds of miles. It shows clearly that life zones are neither latitudinal, nor altitudinal, though these are the two large factors which interact to produce them. We have not discovered an exact mathematical formula for determining life zones on the basis of these two factors, nor are we able to accurately define them on the basis of climatic conditions, but they may be defined and compared in terms of distribution of animals and plants. These, of course, serve as a measure of climatic conditions, that is, as these conditions determine the distribution of the several species. It is apparently not the case that temperature acts in exactly the same way on all species of animals and plants in determining their distribution. For some, it is apparently winter conditions which limit their range into colder regions, though for the great mass, it is probably summer conditions. It is not the purpose of this paper to enter into a discussion of this point, though the fact of winter-killing of certain species is well known, while it is recognized also that every species requires a certain minimum heat budget during the summer, extending over a longer or shorter period, in order that it may reproduce.

It is not to be expected that limiting lines for all species will be exactly parallel, yet there is a large agreement, so that the zones do, in a high degree, maintain their several identities in whatever proportions altitude and latitude interact in producing them. It would be of interest to investigate further into the extent and nature of this identity between the colder zones of the elevated parts of Colorado and the less elevated portions of these zones in the far north. The present study goes merely far enough to demonstrate an agreement of a general nature without showing in detail its extent.

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NOTES ON SOME PROBLEMS OF ADAPTATION.

I. On the Re-formation of the Mantle-glands of Chromodoris.[1]

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I.

Upon the ventral surface of the mantle-fold which encircles the body of nudibranchs of the genus *Chromodoris* there is often found, at the posterior end of the fold, a set of small gland-like nodules producing conical elevations of the skin (Fig. 1). With some species these organs may be absent; in others they are relatively invisible during life, becoming more conspicuous, however, at the death of the animal. In *Chromodoris zebra* the glands during life are usually quite obvious, appearing as a number of white conical bodies rather evenly spaced about the margin of the caudal veil. Here, also, they become more prominent at death, due to increased fluid pressure in the tissue surrounding each gland. A similar degree of enhanced prominence, an enlargement of each conical eminence, is induced by intense stimulation of the nudibranch's skin. The glands apparently act as reservoirs of a repugnatorial integumentary secretion. When the animal is strongly stimulated, the secretion may be seen to flow from the terminal pores of one or more of the conical elevations (Crozier, '16). I refer to these bodies as "glands," loosely; the extent of their truly glandular activity is not yet decided.

The number of glands present on the caudal veil of an individual varies considerably, namely from 0 to 19. In connection with an analysis of the ethology of *C. zebra,* I found it necessary to investigate the physiology of these glands, and to study their numerical variations. From this study certain inferences may be derived as to the nature of the processes leading to the development of the glands. If these inferences be correct, an interesting parallel is afforded with a law of regenerative phenomena in plants (cf. Loeb, '15; '18*a*; '18*b*).

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[1] Contribution from the Bermuda Biological Station for Research. No. 120.

II.

The outline of the caudal veil is characteristically smooth and approximately semicircular. In a certain number of cases, however, there are apparent in this outline obvious irregularities of a secondary nature. Among the individuals presenting a regular, unindented margin, the number of glands varies from 1 to 16 (Fig. 2). The most common arrangement of the glands is, that one is median, with two on either side of it, all evenly spaced (Fig. 1, *B*). Typically, the five glands in such a set are of about the same size, although in many cases the median gland is the largest.

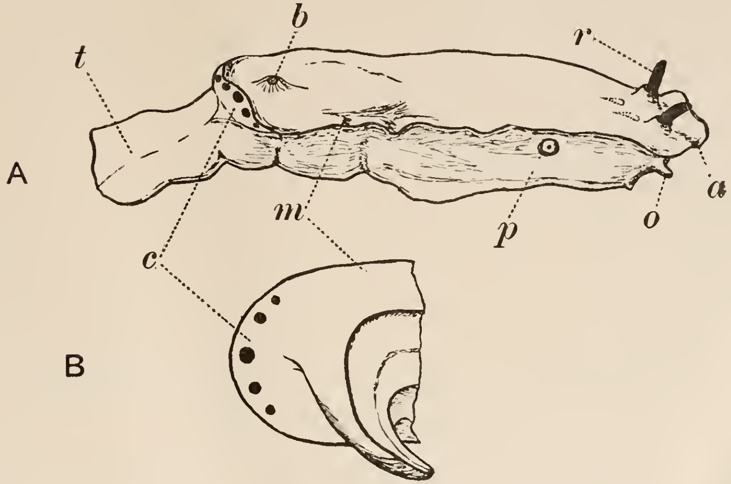

FIG. 1. *A. Chromodoris zebra,* caudal veil (*c*) turned up so as to show the glands (black dots) on its ventral surface; *a*, buccal veil; *b*, branchial collar, contracted over the concealed branchiæ; *m*, mantle folds; *o*, oral tentacle; *p*, reproductive papilla; *r*, "rhinophore"; *t*, "tail" of the foot (× ¾).

B. The caudal veil in ventral aspect, "tail" part of foot turned to one side; this shows a plan of distribution that, on the whole, is the most common.

Although five is the modal number of glands (Fig. 2), other numbers are common; some of these arrangements are illustrated in Fig. 3. Not infrequently one or another gland in an otherwise symmetrical distribution seems omitted, or suppressed (Fig. 4). It seems possible, if not probable, that each gland may undergo a more or less cyclic sequence of growth and shrinkage.

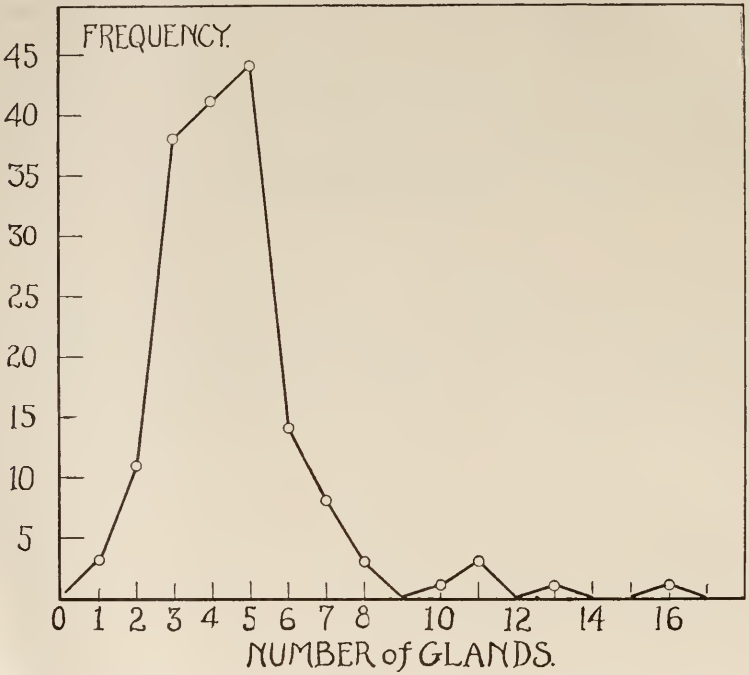

Fig. 2. Showing the frequency with which different numbers of glands occurred in a series of 166 individuals; no obviously irregular sets of glands were included (see text).

Independently of such variations, there is a still further diversity of numbers and sizes among the different sets. As to the number of glands, some examples are given in Fig. 3. In the instances illustrated, and indeed throughout series of several hundred animals each, examined at different times since 1915, it is generally true that if the number of glands in a set is low, their size is individually larger than in the case of groups composed of greater numbers. Within certain limits, which do not concern

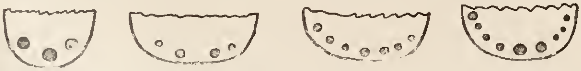

Fig. 3. Examples of symmetrical sets of "glands" (× 2/3).

the series of individuals here involved, this is true without reference to the sizes of the nudibranchs. As shown by Fig. 5, the number of glands present in symmetrical sets is independent of the size of the animal.

This leads to the idea that, in general, only a definitely limited amount of material is available in each animal for the formation of the white mantle-bodies, regardless of the *number* of these bodies which may be present.

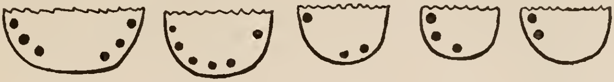

Fig. 4. Examples in which one or more "glands" are omitted (× 2/3).

LENGTH, CMS.

NUMBER OF GLANDS.

	3	4	5	6	7	8	9	10	11	12	13	14	15	16
0							1							
1						1	1							
2					1	1	3	2	4	1				
3			2	2	8	6	6	7	7					
4	1		1	1	4	8	11	6	7	2	3			
5		2	1	5	13	13	11	6	5	2	3		2	2
6	1				1	7	5	5	2		2			
7				2	1	2	3	2						
8							1		2					
9														
10				1		1								
11								1						
12														
13							1							
14														
15														
16							1							

Fig. 5. Showing the absence of sensible correlation between the *number* of glands on the caudal veil of *Chromodoris* and the *size* of the nudibranch (203 individuals), among instances exhibiting a normal symmetrical distribution of the glands. The column of modal length and the row of modal frequency of glands are enclosed between parallel lines.

III.

A frequency-distribution of the numbers of glands found on different individuals has been given in Fig. 2. In order to avoid certain non-pertinent complications, perhaps resulting in part from seasonal differences (Crozier, '19*a*), I have included in

Fig. 2 only data from one series, obtained September 21–30, 1918. In addition to the individuals involved in Fig. 2, 17 *Chromodoris,* or 9.3 per cent. of the total collected at this time, were seen to have the margin of the caudal veil markedly irregular. Instances of this sort are shown in Fig. 6.

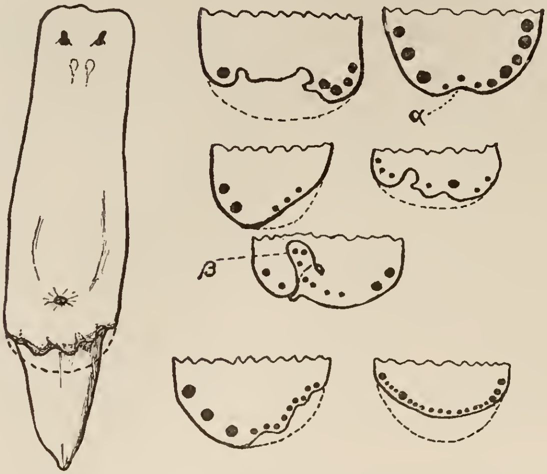

FIG. 6. Showing the character of injuries suffered by the caudal veil, and the results of removal of glands; at *α*, probably one gland had been removed; at *β*, a tongue of torn tissue; original outlines approximately as indicated in dashed lines (× 2/3). See text.

It has nearly always been true in cases of marked irregularity of the caudal mantle that several small glands are situated at points where but one such organ, and that a large one, would characteristically occur. This has been interpreted to mean that if, as the result of injury, one or more glands have been removed, material constantly being transported to these regions is then collected into a greater number of small glands.

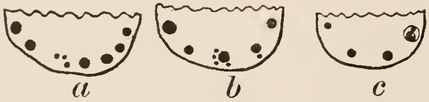

FIG. 7. In *a*, three small glands (surrounding the location of a shrunken gland?); at *b*, a condition which may result from the subsequent enlargement of a gland much shrunken for a time; at *c*, the inclusion (?) of a small gland by an enlarging one normally situated (× 2/3). See text.

It is in fact known that injuries are suffered by the mantle-fold and by other parts of *Chromodoris,* mainly (if not entirely) through the biting of fishes (cf. Crozier, '19*a*). The re-formation of the caudal veil, and with it of mantle-glands, after amputation, has been observed in the laboratory. The process was slow, however (Crozier, '16), occupying some 4 months, and was therefore unsuitable for experimental study; the tissue of the mantle-fold is regenerated very slowly indeed, new glands appearing before an excised region of the caudal veil is restored.

The view here advanced involves the idea that the major portion of the gland-contents, or the precursors of these contents, are in some way carried from distant parts of the animal to the site of the glands. It can be pointed out that the repugnatorial substances contained in the glands are indeed present in the general mantle surface of the nudibranch. Several instances were noted wherein a nudibranch with a much injured caudal veil exhibited 3 or 4 small white accumulations of secretion on the ventral surface of the *buccal* veil. A detailed account of these secretions, with proof of their repugnatorial character and function, I shall provide in another publication.

Microscopically, each gland consists of a spherical sac, tightly packed with oily globules of a special kind, and communicating with the outside by means of a minute pore. In *C. zebra* the histological conditions are not unlike those earlier figured (somewhat incompletely) by Bergh ('98) for *C. juvenca.* Bergh did not observe the pore-like opening, which is difficult to see in sectioned material, and indeed is absent save in mature glands, and at this time the real nature of these structures was unknown. On some other portions of the mantle fold, notably on the ventral surface of the buccal veil, minute white bodies are occasionally seen, which give rise to a similar secretion; these bodies are never so large as the caudal glands.

It has been remarked that each gland may perhaps undergo a process of shrinkage, followed by growth to a maximum size. This might explain such cases as are illustrated in Fig. 7. Materials ordinarily going to a particular gland might, if this gland be in a phase of shrinkage, accumulate in several smaller, new glands about its periphery; subsequent re-growth of the original gland could then result in a condition such as is shown in Fig. 7, the further development of the new glands being prevented.[1]

IV.

Loeb ('15, '18*a*, '18*b*) has sought to account for certain fundamental characteristics of organ-formation, as seen in *Bryophyllum,* on the basis of a flow of "organ forming" materials. In the case of buds arising from notches in an isolated leaf of this plant, the buds first growing out attract to themselves materials available in the leaf for the growth of buds. The total amount of such materials being limited, the growth of these first buds automatically inhibits the development of others. This explanation has the advantageous support of really quantitative experiments, and it is of interest to notice how closely it may apply to the not very dissimilar phenomena of gland re-formation on the caudal veil of *Chromodoris.*[2]

Regeneration experiments have made it probable that the supply of materials to the region of the glands is so slow that over any given period the total available quantity of such material may be regarded as fixed. The relative sizes of the several glands in a set, in cases where size differences are evident, show that a certain proportion of the gland materials is normally received by each of the (commonly 5) regions of the caudal veil where a gland is characteristically developed. So long as the original glands are present in full activity, no further formation of glands seems to take place. If, however, some of these be removed, the current of secretory materials continues to this region of the mantle and results in the formation of an excessive number of new glands, which individually remain of relatively small size.

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[1] A distinct tendency is noticeable for the more anterior glands of symmetrical sets to be smaller than the medial ones, pointing to a bilateral scheme for the distribution of the pro-secretory materials. The exact conditions attending the development of a gland at a particular place can be made clear only from histological study. It seems probable that any portion of the epithelium of the caudal vein *can* give rise to a gland.

[2] Some perhaps analogous phenomena have been described in the growth of previously injured Madreporarians, where normally there is a "dominant apical zooid" (cf. Wood-Jones, '12, p. 112). The manner in which exhausted gland-cells of the amphibian skin are replaced by the enlargement of previously "dormant" cells is also suggestively similar.

The size relations make it clear that what might loosely be regarded as an adaptive over-production of repugnatorial glands subsequent to injury, probably results, on the contrary, from (1) the limited quantity of the repugnatorial precursor substances, and (2) the fact that normally the development of new glands is inhibited through the attraction ("drainage") of these substances by glands already established. This will also explain why in cases where, for example, but 3, rather than 5, "glands" are present on an undamaged mantle, the "glands" are in the former instance individually larger. What it is that determines the number of glands originally formed, remains undiscovered; they are probably absent in all individuals of *C. zebra* less than 1 cm. long.

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NOTES ON SOME PROBLEMS OF ADAPTATION.

2. On the Temporal Relations of Asexual Propagation and Gametic Reproduction in Coscinasterias tenuispina: with a Note on the Direction of Progression and on the Significance of the Madrepores.[1]

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I.

Some time ago I gathered evidence showing that in the common Bermuda starfish, *Coscinasterias tenuispina* (Lamk.), there is unmistakable evidence of asexual multiplication through spontaneous division of the body into two parts (Crozier, '15). The modal number of rays in the adults of this species is 7[2]; the parts resulting from self division commonly constitute either 3 or 4 rays; each of these parts most usually produces 4 new rays. I may give here some additional data obtained in subsequent years, leading to the same conclusion (Fig. 1, Table I.).

Table I.

The Relation between the Number of Long and the Number of Short Rays in 237 Coscinasterias, of which Each Individual had Two Distinct Groups of Rays.

The table shows that as a rule 4 short rays are found in association with either 3 or 4 long rays.

LONG RAYS

SHORT RAYS.	1	2	3	4	5	6	7	
1			1	1	5	5	3	15
2			6	5	8			19
3		2	18	32	5			57
4		8	42	55	4			109
5		7	17	7	1			32
6	1	3			1			5
	1	20	84	100	24	5	3	237

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[1] Contributions from the Bermuda Biological Station for Research. No. 121.

[2] It is entirely probable that here, as in *Solaster* (Gemmill, '12) and in *Leptasterias* (Osterud, '18), the fundamental ray number is 5, to which at an early age a sixth and, later, a seventh ray are normally added (cf. Crozier, '15).

The ordinary, gametic reproduction of these starfishes takes place in January–February. During summer months they are quite abundant under stones along the shore, just under low-water level. As winter approaches, they retreat for the most part to somewhat deeper water, it would appear. In January and February, however, they crowd up to high-water mark, even on exposed shores, within Great Sound at least, and in Harrington Sound.[3] Often they are consequently left high above water level

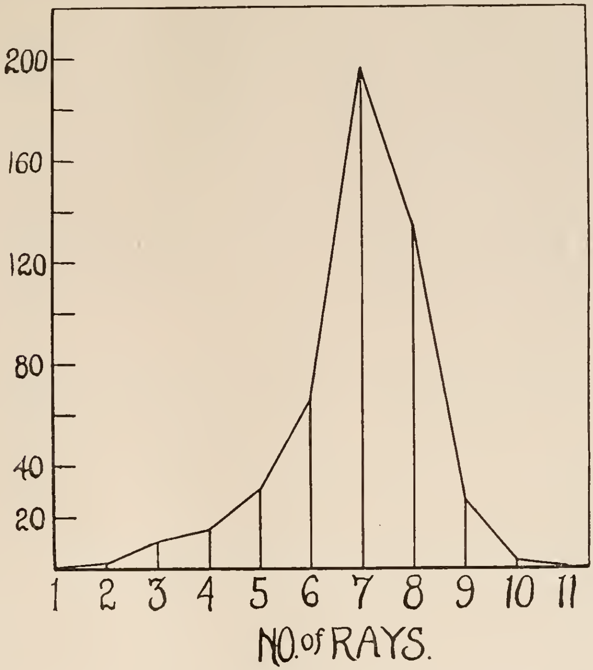

FIG. 1. The ray-frequency polygon derived from counts of 487 *Coscinasterias.* The essential characteristics of this curve are identical with those of a curve previously published (Crozier, '15).

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[3] The question presents itself as to the possible rôle of phototropism in such shoreward movements. *C. tenuispina* is photonegative toward strong light, photopositive toward weak light; it is probable that these animals become photopositive toward slightly greater illuminations with lower temperatures. This might play some part in determining movements into more illuminated areas during the winter season; presumably gonadic secretions might also be involved. Plessner ('13) came to the conclusion that the "eye-spots" of starfishes enable these creatures to respond to the *direction* of light; in *C. tenui-*

when the tide recedes. Since they are not strongly photopositive, they are seldom exposed to the drying action of the sun, and survive the temporary absence of water. Occasionally they have been found killed by rain (this has also been seen with *Stichopus mœbii*).

I always noticed that in the breeding months the proportion of animals which seemed to have recently undergone self division was much less than during the summer. Some attempt was therefore made to discover the percentage of recently divided specimens obtainable in each month of the year. This was done, somewhat unsystematically, for several years. The result is set forth in Table II. The data for each two-month period might have been averaged together, thus perhaps obliterating to some extent the possible variation from year to year. The numbers in the last column of the table express for each period the percentages in which the rays were 5 to 9 in number and of approximately equal length—in other words, those which exhibited no evidence of reasonably recent self division.

Table II.

The Percentage of "Non-regenerating" Coscinasterias Secured in Random Collections During Each Month, 1915–1918.

Period.	Total No. of Individuals	Not Recently Divided,—*Per Cent.*
January	74	59
February	60	63
March	35	60
April	30	40
May	34	17
June	18	11
July	113	12
August	74	33
September	40	20
October	32	41
November	27	33
December	20	75

For June–July–August, 1913 and 1914, I found (Crozier, '15) 83.6 per cent. of the individuals to exhibit a group of longer and

spina, however, the tips of the rays shorten noticeably when a shadow is cast on them alone, whereas in sunlight the tips of the rays tend to be curled upward.

a group of shorter rays. In the same months of 1915–1918, the percentage was 80.4, a sufficiently good agreement. This is about the maximum percentage of regenerating, or recently divided, individuals encountered during a twelve-month period. From Fig. 2 it is evident that the highest percentage of intact specimens not recently divided occurred during December to March—the percentage then rather rapidly sinking to a minimum during midsummer.

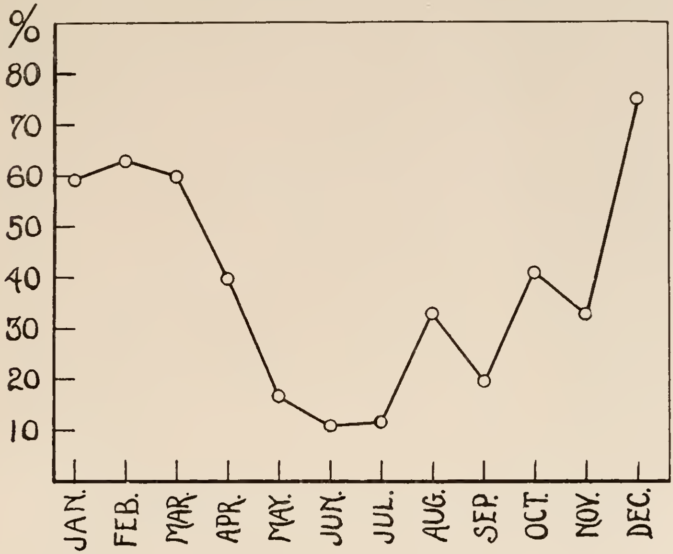


Fig. 2. The average percentage of *Coscinasterias* not showing evidence of recent self-division, during each month (1915–18).

There is a certain correlation between the course of the curve of self-division (Fig. 2) and that of temperature-variation in the sea water. I reproduce here a seasonal temperature graph derived from figures given by Verrill (1902), together with the averages of my own data; the curves agree rather well, in spite of the fact that my temperature readings were not very numerous, nor systematically taken, but were obtained mainly for different special purposes having to do with other problems.

In comparing Figs. 2 and 3, it must be recalled that the "curve of self-division" is non-quantitative in one important respect: the percentages of dividing animals include data derived from individuals which may have divided some little time previous to others, as indicated by the various stages in regeneration classified under this head. A "lag" in this curve of division with respect to water temperature might therefore be expected. The sudden rise of percentage of "non-regenerating" specimens at December, however, is probably adequate proof that a month or so is sufficient time for the full growth of new rays. The actual "lag" in the curve of autotomy is of the wrong sort for the idea of complete temperature control, because the decrease in frequency of self-division perceptibly precedes the seasonal decrease of temperature. This might be explained on the basis that the onset of higher temperature in spring serves indirectly to induce self-division, but that the capacity for this method of propagation is gradually exhausted, or in some fashion checked, before the warmer season has closed.

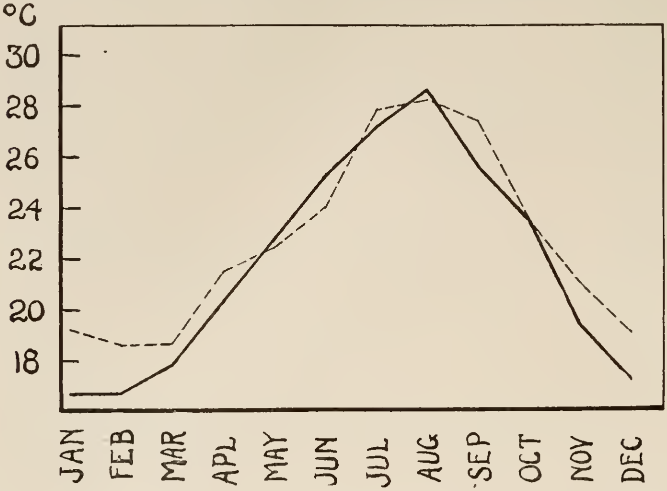
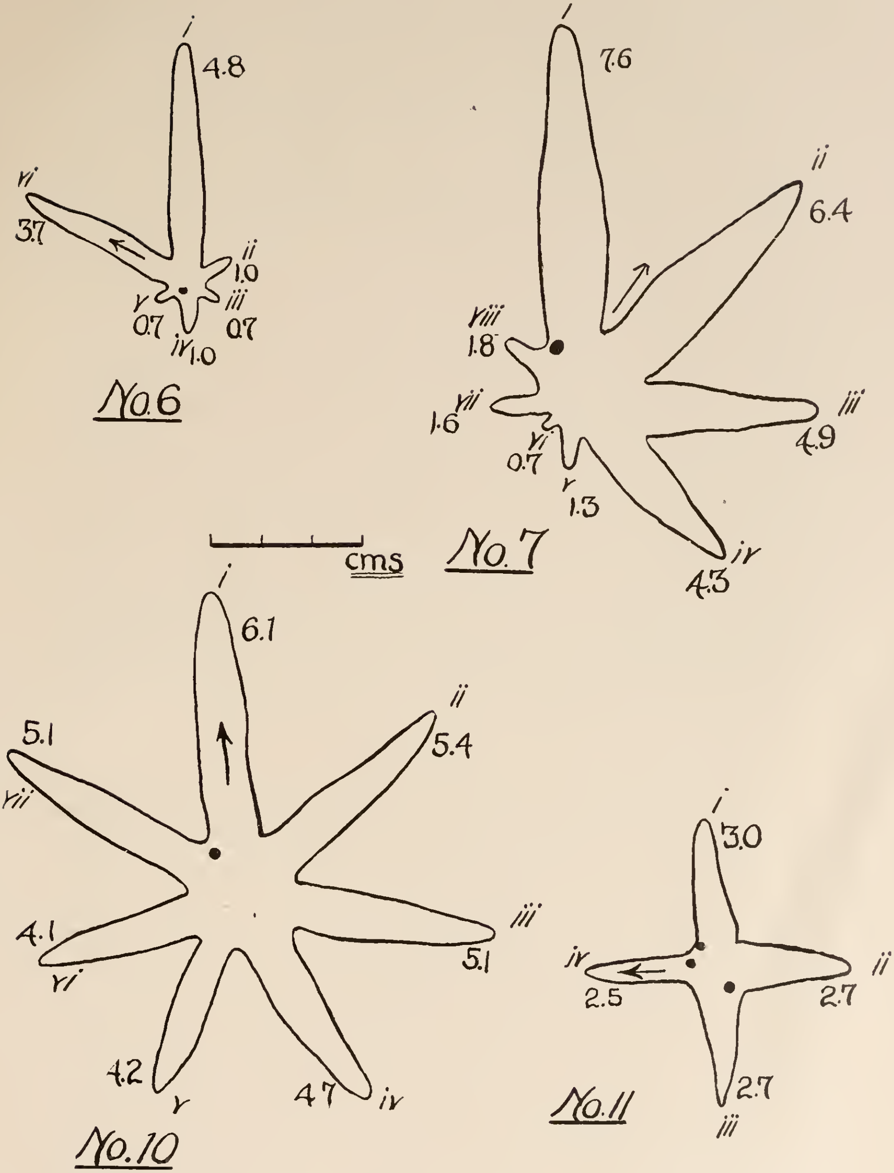


Fig. 3. Mean monthly temperatures of seawater at Bermuda. Heavy line, data from Verrill ('02); dotted line, averages from records of W. J. C. The dotted line records for winter months are higher than those given by Verrill, probably because my own observations were restricted for the most part to the waters of the partially enclosed Sounds.

It is interesting, however, to notice how the frequency of self-division is enormously decreased at the approach of the period of breeding. There is thus accomplished, whether through the


i
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vi
3.7
ii
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iii
0.7
v
0.7
iv
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No.6
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viii
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vii
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vi
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v
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iv
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3.0
iv
2.5
ii
2.7
2.7
iii
No.11

Fig. 4. The direction of progression (arrow) in two typical instances of recently autotomized halves of *Coscinasterias*. *a*, division surfaces; non-directive stimulation.

agency of temperature or in some other way, a nice adjustment of the two dissimilar methods of multiplication practiced by this species. Asexual increase through self-division of the body is at a minimum just before and during gametic reproduction. It is not difficult to suppose that an undivided individual is better suited for gamete formation and for such coördinated movements as probably are concerned in bringing the sexes near together for fecundation of the eggs. The general energy of the animal is then directed toward the elaboration of gametes, rather than diverted to the formation of new tissue. To what extent the elements of this correlative mechanism are causally related, however, one must refrain from guessing. Judging from the statements of Clark ('13*a*, '13*b*) and Mortensen ('17) regarding the asexual multiplication of *Linckia guildingii,* and the period of its egg-reproduction, I suspect that the supplementary relation here pointed out for *Coscinasterias* may not obtain with *Linckia,* where, however, the method of self-division is different, consisting not in the division of the body across the disc but in the abstriction of a portion of a ray. Nor in *Holothuria surinamensis* is there good evidence of an alternation of the sexual and asexual methods of multiplication (Crozier, '17).

II.

The number of madrepores in *Coscinasterias* is quite variable, from 1 to 5 being found on a disc. As earlier suggested (Crozier, '15), the number of madrepores is correlated rather definitely with the total number of rays (Tables III., IV.). It was observed that during the addition of new rays, subsequent to self-division, one or more new madrepores are also added. In almost every case, throughout the several hundred carefully examined, each moiety of a divided body bore one or several "old" madrepores. Moreover, some of the very largest specimens collected, with ray lengths of 12–13 cms., had but 5, 6, or 7 rays, of equal length, with but *one* madrepore. These facts suggested that the development of several madrepores is perhaps a more or less necessary preliminary to normal self-division. The madrepores are usually formed at widely separated points on the periphery of the disc; hence the probability is that each part of an animal, when the creature divides, will carry at least one of these bodies.

Table III.

The Correlation between Number of Madrepores and Number of Rays in a Random Sample of 275 Coscinasterias.

Note that one or several madrepores are always present on the parts of recently bisected (autotomized) individuals.

NO. OF RAYS.

MADREPORES.	2	3	4	5	6	7	8	9	10	
1	2	5	4	5	13	21	10	2		62
2		2	4	5	10	25	14	3	2	65
3					6	29	30	2		67
4					5	18	28	4		55
5				1	1	2	4			8
	2	7	8	11	35	95	86	11	2	257

Table IV.

The Correlation between Number of Madrepores and Number of Rays, Among 82 Coscinasterias Selected so as to Include Only Individuals in which all the Rays were of Very Nearly Equal Length.

One or several madrepores commonly appears at the edge of the disc where rays are being newly added, subsequent to a self-division.

NO. OF RAYS.

MADREPORES.	3	4	5	6	7	8	9	
1	4	2	3	5	11	1	1	27
2	1	2	3	2	5	2	1	16
3		1		2	14	6		23
4				3	8	4	1	16
	5	5	6	12	38	13	3	82

Cole ('13) discovered that *Asterias forbesi* tends to creep in such a way that, in the absence of directive stimuli, the portion of the animal nearest to the single madrepore is usually in advance—a true, though subordinate, "physiological anterior." He suspected that the water-system might be concerned in some way with the determination of this "anterior." The conditions in *Coscinasterias* might perhaps be regarded in one, or both, of these two ways: (1) there is a physiological basis for the development of one or several madrepores according to the number of rays, *i.e.*, depending on the total water requirements of the locomotor organs; or (2) the multiplicity of madrepores is adaptively related merely to the habit of self-division. At first I was inclined to entertain the former idea; the fact, however, that 7-, 8-, and 9-rayed individuals, as well as those of 5, 6, or 7 rays and of large size, do get along quite well with a single madrepore is more favorable to the second notion.

I would regard it as possible, then, that the multiplication of madrepores at separated points on the disc of *Coscinasterias* furnishes merely an assurance that portions of the body separated by autotomy will each be provided with a madreporic canal. It is not improbable, however, that the very development of supernumerary stone-canals, by furnishing each an additional "physiological anterior point,"—such as that revealed by Cole's ('13) experiments with *Asterias*,—provides automatically the very conditions determining self-division.

III.

The probability of this explanation of the significance of the madrepore might be tested by determining the direction of progression in various individuals, under non-directive stimulation. I studied from this standpoint the locomotion of 12 *Coscinasterias*. Non-directive conditions were secured very much as in Cole's ('13) experiments: an 8-c.p. filament, the only source of light, was suspended in the axis of a circular aquarium tub 3 feet in diameter, and 4 feet above the surface upon which the starfish crept. The tub held sea water to a depth of 10 cms. One half hour elapsed between trials. The observations were made at night. To horizontal light of this intensity, *Coscinasterias* was slightly photonegative, but more intense illumination was required to cause decided negative movements. Even under the supposition that illumination of a larger ray might lead to the determination of directed photonegative movements when the animal is illuminated vertically, owing to the larger sensory surface of such a ray, the conditions here established must be regarded as essentially non-directive, because the starfishes often crawled with some other than the longest ray *in advance*.

The length of the rays and the number of madrepores presenting such great variations, it was possible to obtain a fairly definite idea of the relative values of *ray-length* and *madrepore-position* as determiners of the "physiological anterior"; for *Coscinasterias* does show definite tendencies in the orentation of its body with respect to creeping. Since the organization of the body of this starfish is somewhat irregular, the observations cannot be summarized in terms of morphologically defined rays (I did not note the position of the anal opening in these cases; cf. Gemmill, '14). I must therefore give several instances in detail (Table V.).

TABLE V.

SHOWING THE NUMBER OF TIMES EACH RAY OF THE STARFISHES ILLUSTRATED IN FIGS. 4 AND 5 WAS USED AS "DIRECTOR."

No. of the Animal.	No. of Trials.	Ray.								
		i.	ii.	iii.	iv.	v.	vi.	vii.	viii.	ix.
1	33	8	18	0	5	2				
2	24	10	10	1	1	1	1			
3	30	8	3	2	0	1	8	2	0	6
4	16	15	0	0	0	0	1			
6	16	1	0	0	3	0	12			
7	15	7	8	0	0	0				
10	16	8	0	0	0	0	4	4		

In the creeping of *Coscinasterias,* which is rather slow (at 24°, about 12–15 cms. per min.), several points are similar to ones emphasized by Cole in *Asterias,* notably the tendency to exhibit a definite region as "physiological anterior," with, however, clear evidence of the "rotation" of this anterior.[4] A good instance of such rotation is the following:

Coscinasterias No. 1, September 9, 1915. Creeping under vertical light, the region in advance shifted slowly, in this order (cf. Fig. 5): i, i, ii, ii–i, ii, ii, iii, iii–iv, v–iv, the animal being undisturbed from the outside.

Such "rotation of the impulse" was observed to be either clock- or anticlockwise. Ordinarily rotation of this sort was not conspicuous, so that a fairly definite region could be distinguished as an "anterior" in creeping. From the typical records submitted, it appears that as a rule either the longest ray, or a long ray near a madrepore, is the "directive" ray.

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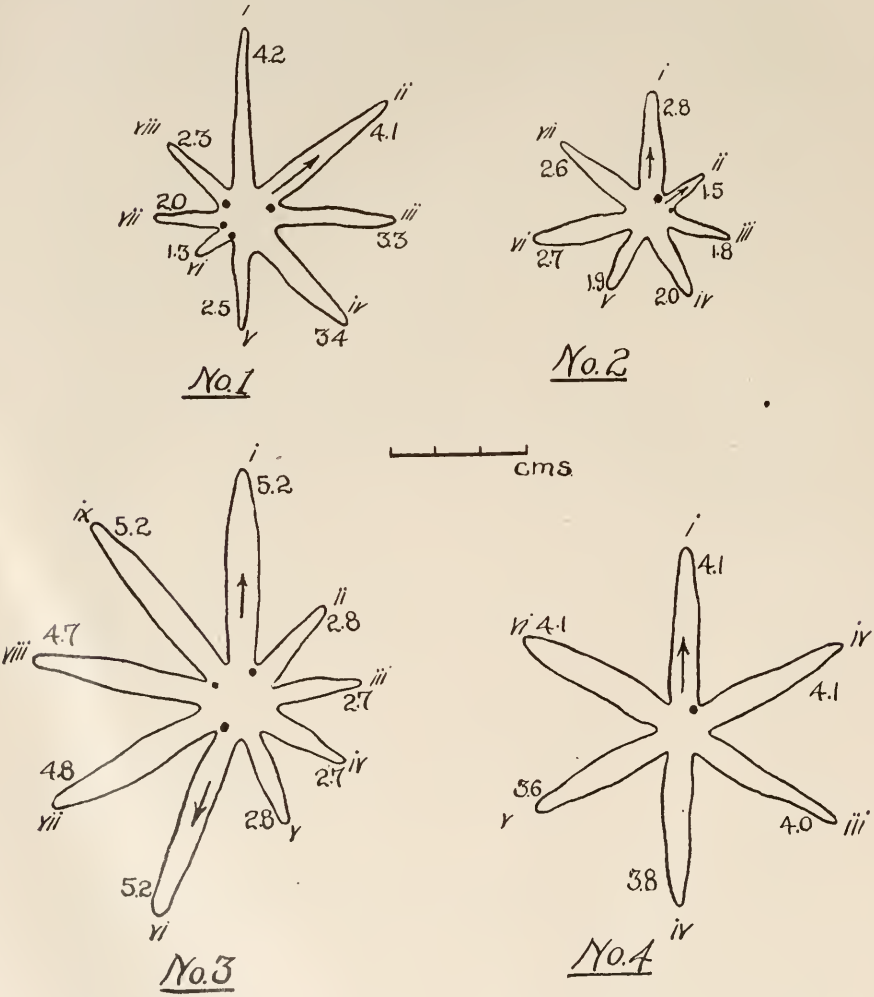
[4] Agersborg ('18), in describing the righting movements of the "twenty-rayed" *Pycnopodia helianthoides,* has spoken of "bilateral tendencies" in the activities of this starfish. But the condition of his tests are not well specified, nor is there any indication given of a relation between the "bilaterality" and structural conditions in *Pycnopodia.*

As to the relative importance of ray-length and position of madrepore, it seems clear that, atlhough no absolute rule can be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vi
No. 3
i
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vi
4.1
iv
4.1
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v
4.0
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No. 4

Fig. 5. Figs. 4 and 5 show typical instances of the "physiological anterior" in *Coscinasterias* (see Table V); *i, ii,* . . ., numbers assigned to rays; lengths of rays in cms.; madrepores, black; predominant directions of locomotion under non-directive stimulation shown by arrows.

stated, the latter factor is, on the whole, preponderant.[5] In 13 cases, where the disposition of rays and of madreporites was most favorable, the *longest ray* was recorded as " director," in the average, for 32.2 per cent. of the trials; a *ray near a madrepore* being so recorded, in the average, for 42.2 per cent. of the trials.

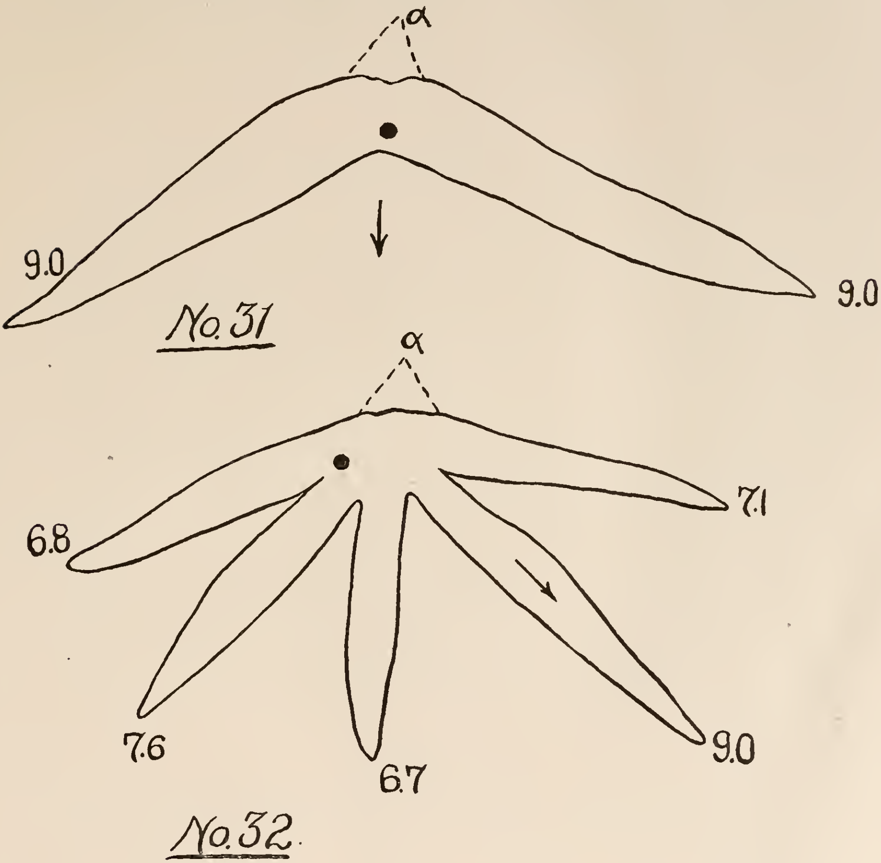

Fig. 6. The direction of progression (arrow) in two typical instances of recently autotomized halves of *Coscinasterias.* α, division surfaces; non-directive stimulation.

More convincing than such averages, however, are the actual data already set forth.

Frequently, in the case of animals having two ray-groups greatly differing in size, the directive ray appears to be one next to the plane of antecedent autotomy. This fact might seem unfavorable to the conception of the "cause" of autotomy above formulated, but in fact I found that if the creeping of halves of starfishes recently separated was studied, the results were, on the contrary, favorable to this conception. Examples in point are afforded by such specimens as are illustrated in Fig. 6.

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[5] In connection with the matter of physiological and anatomical polarity in echinoderms, compare Clark's observations on the locomotion of *Comatula purpurea* ('15, p. 112), and mine on the creeping of *Mellita* (Crozier, '20); in both forms there is clear evidence of functional bilaterality.

IV.

Summary.—It has been pointed out that in those individuals of *Coscinasterias tenuispina* which divided autotomously more than one madrepore is developed on the disc previous to division. Further, that each madrepore, or group of madrepores, is an external sign (at least) of the development of a tendency toward the establishment of a "physiological anterior" point, as expressed in creeping. Hence it is suggested that the cause of the autotomous separation of the body of this starfish into two approximately equal parts—each as a rule then automatically receiving one or more madrepores—may be intimately connected with the very development of these structures. It is shown that this asexual multiplication by self-division exhibits a seasonal rhythm so adjusted as to constitute a propagative method which supplements, in point of time as well as in kind, the ordinary gametic reproduction, the former method being distinctly in abeyance during the winter months (January–February), when breeding occurs.

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NOTES ON SOME PROBLEMS OF ADAPTATION.

3. The Volume of Water Involved in the Cloacal Pumping of Holothurians (Stichopus).[1]

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It has been of interest, especially in connection with studies of respiration, to determine the volumes of seawater which various marine animals pass through their respiratory organs. With the sponge *Spinosella* Parker ('14) calculated from his measurements that something like 78 liters a day was the volume forced through a single "finger" of the body of this sponge. For *Ascidia,* Hecht ('16) showed that the branchial stream was probably somewhat in excess of 173 liters per day, in individuals of medium size. The water current in such cases (the only ones of the kind carefully investigated) brings not only oxygen but food particles as well, and in addition has an excretory significance.

I undertook to measure the volume of seawater involved in the cloacal pumping of a holothurian such as *Stochopus mœbii,* in which water is forced into respiratory trees and then expelled to the exterior. The cloacal chamber of *Stichopus* pulsates with a frequency which depends on the size of the animal; after a certain number of pulsations the water contained in the respiratory trees is forced out; the number of pulsations between two acts of "spouting" also depends on the size of the individual (cf. Crozier, '16). As in all such cases, the expelled water is forced out rather violently, driven to a considerable distance, so that it is not readily taken in again when inspiration is again begun (Hecht, '16; Arey and Crozier, '19).

By previously ascertaining the mean number of inspiratory pulsations elapsing between "spoutings," it was possible to remove a given *Stochopus* from the water just before the beginning of an expiratory act, and to receive the discharged water in a funnel leading to a graduated cylinder. Employing 10 individuals each 24–25 cms. in length, I found an average volume of 15.5 c.c. of of seawater to be expelled in this way.

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[1] Contributions from the Bermuda Biological Station for Research. No. 122.

If a cut through the body-wall be made rapidly around the body of a *Stichopus* at the level of the anterior end of the cloacal chamber (Crozier, '16), it is possible to obtain a preparation in which the respiratory trees remain intact and preserve their normal connection with the cloacal wall. Such a preparation will live for a long time in seawater, and it can be seen that water is pumped, as normally, into the respiratory trees, and that when these organs have been expanded to a certain degree their contained water is expelled in the usual manner. In this process the respiratory trees themselves are actively contractile (cf. Iwanzoff, '97; Henri, '03); they shrivel up almost, but not quite, completely —so that nearly all their contained water is expelled. The independent nature of the activity of the cloaca and respiratory trees affords a curious instance of the autonomy of the organs in echinoderms.

Examination of the interior of an intact *Stichopus* at the conclusion of "spouting" showed that the respiratory trees were in this case also almost completely contracted.

The cloacal chamber of *Stichopus* 24.5 cm. in length pulsates with a frequency of 9.6 per minute (at 27°), and 8 or 9 inspiratory pulsations intervene between two expirations, each of the latter occupying some 12 seconds (cf. Crozier, '16). On this basis a *Stichopus* of this size will (at 27°) take into its cloaca about 15.5 c.c. of water during each 65 seconds, or about 859 c.c. per hour; leading to an estimated amount of 20–21 liters per day, assuming a uniform rate during the 24 hours.

From the comparative standpoint, it must be remembered that probably little or no food-getting is associated with the entrance of water into the body of *Stichopus* by way of the cloaca, Pütter's ('07) notion to the contrary notwithstanding; but that, in contrast to the situation in sponges and in ascidians, respiration and excretion are in holothurians the sole functional implications of the water stream. In this way it can be understood that the volume of water required by these animals is much less, even in proportion to their size, than is the case with sponges, lamellibranchs, or ascidians.

The respiratory value of the water moved by the cloacal pump is clearly evidenced by this fact: seawater in which a *Stichopus* was living had a reaction of pH 8.2; the pH of the water expelled during spouting was 7.8; that of the fluid within the body-cavity, 7.6. It is obvious that even within a minute's time relatively considerable quantities of CO_2 are able to diffuse across the thin membraneous wall of the distended respiratory trees. According to Winterstein ('09) about 50 per cent. of the respiratory loss of CO_2 is accomplished through the "trees."

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BIOLOGICAL STUDIES ON INTRACELLULAR BACTERIA.[1] NO. 1.

R. W. GLASER.

INTRODUCTION.

It has been known for a long time that certain blattids, homoptera, and ants harbor intracellular non-pathogenic organisms that are transmitted from generation to generation through the egg. Since Huxley's description of the pseudovitellus of aphids in 1858 a host of investigators have studied the embryonic development and morphology of this curious organ and its contents. After Huxley, the most prominent investigators associated with the study of this subject and with the development of the entire field of intracellular organisms in insects, were Lubbock, Metchnikoff, Balbiani, Krassilstschick, Blochmann, Lindner, Heymons, Berlese, Mercier, Pierantoni, Sûlc, Buchner and others.

This vast study has precipitated the following facts and theories which may be presented seriatim.

1. The insects that are definitely known to harbor intracellular non-pathogenic organisms belong to the family Blattidæ, the entire order of Homoptera, and the family Formicidæ.

2. It has also been reported that certain Lepidoptera and Coleoptera harbor intracellular non-pathogenic organisms, but conditions are not clear in the cases cited and the authors may have been confronted with true pathologic cases.

3. In the insects where non-pathogenic intracellular residents have been established one or more definite species of microorganisms are always associated with every individual of a particular species of host.

4. The microörganisms associated with the insects have been called symbionts.

5. The symbionts are transmitted from generation to generation through the egg in a very definite manner.

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[1] Contribution from the Entomological Laboratories of the Bussey Institution, Harvard University. Bussey Institution No. 174.

6. In adult insects the symbionts are sometimes found free in the lymph, but in most species are found enclosed in very definite, modified cells known as mycetocytes or bacteriocytes.

7. In some species two or more mycetocytes fuse and become concentrated forming a very definite organ known as the mycetome. Some insects are monosymbiotic, that is, they harbor only one species of symbiont; others are disymbiotic or trisymbiotic.

9. When the host is monosymbiotic the symbionts may be found in mycetocytes or may be concentrated in a mycetome.

10. When the host is disymbiotic one species of symbiont may be found in mycetocytes and the other in a mycetome, or both symbionts in separate mycetomes or both symbionts in separate feebly connected mycetomes or lastly both species may be in one mycetome.

11. When the host is trisymbiotic we may have two species of symbionts in one mycetome and the third in mycetocytes or the three symbionts may be in one mycetome.

12. The host cells show specific changes, but no injury. They usually enlarge. If the symbionts are small the cytoplasm has the appearance of being finely reticulate; when large the cytoplasm becomes coarsely reticulate. The nuclei of the host cells are sometimes round; at other times curved or even arborescent. The organisms never invade the nuclei. In spite of deformation of the cytoplasm, the nucleus retains its ability to divide. Sometimes one finds nuclear and cytoplasmic degeneration, but this is an exception and probably due to some secondary parasite or to disease.

13. Most of the mycetocytes are enormous in size when compared with the remaining cells of the animal.

14. The systematic position of the symbionts is not always clear. In the case of the Blattids they are undoubtedly bacteria; in the case of some aphids undoubtedly yeasts, and in the other cases yeast-like organisms and probably higher fungi. No protozoan intracellular symbionts of insects are known.

15. The symbionts from a small number of host species have been cultivated upon artificial media, but in most cases the artificial cultivation experiments of symbionts have so far met with unsurmountable difficulties. This may be due to the fact that

the exact conditions existing in the mycetocytes or mycetome have not been sufficiently reproduced.

16. The number of symbionts per host is enormous. For *Ceroplastes rusci,* Berlese computed the number per host to lie between sixty and seventy thousand.

17. The reasons for the belief that the intracellular organisms are symbionts and not commensuals or parasites are the following:

(*a*) Every individual of a species is infected.

(*b*) The infection produces changes in the host cells, but these are harmless.

(*c*) The infection routes and methods of localization, while different in different hosts and symbionts, follow very definite courses within a species.

(*d*) The microörganisms are numerically controlled by the host, never increasing up to a point where they may prove fatal.

(*e*) The microörganisms within the insects obtain nourishment, and protection from drastic temperature and draught conditions.

18. While numerous theories have ben advanced, the symbiotic relationship, if any exists, has not been established upon any scientific basis.

Buchner (1912) in an extensive paper, together with original morphological investigations, gave an excellent review of all of the literature dealing with the subject of the intracellular symbionts of insects. For this reason, another extensive review of the literature would be merely a duplication of something that has already been admirably accomplished.

In beginning this investigation, I was primarily interested in the physiological aspect of the subject. If the intracellular organisms are really of some use to the host, they may possibly assist in the metabolism of reserve and other foods. This can only be accomplished through the secretion of enzymes by the microörganisms. I therefore thought it well to select one or two species of insects, to attempt to cultivate the intracellular organisms, then to make a systematic study of their enzyme activities, and later to attempt to correlate such activities with the metabolism of the host's diet.

For the above work, I chose two species of cockroaches for the reason that conditions seemed simpler. Cockroaches can be easily obtained and reared. They are large insects when compared with aphids, coccids, psyllids, aleurodids, etc., and can be readily dissected. Moreover, Mercier (1906) reported that he had cultivated the so-called "Blochmann bodies," thus putting an end to the tiresome discussions of Cuenot, Prénant and Henneguy, who supposed that the rods were metabolic products and not bacteria. Mercier cultivated these organisms on nutrient agar, gelatine, potato, milk and bouillon. He described only the morphological characters and named the organism *Bacillus cuenoti.* The fact that one species of cockroach had yielded an organism that could be cultivated also led me to choose these insects in preference to others. Lastly, cockroaches are monosymbiotic; in other words, conditions are not, so far as known, complicated by the presence of more than one microörganism.

Before proceeding to the cultural and physiological section of this paper it might be well to glance over the morphological results in Blattids as shown by Blochmann, Heymons, Buchner and others.

1. The Bacteriocytes and Bacteria in Blattids.

In *Blattela germanica* and *Periplaneta orientalis* certain of the fat cells are filled with bacteria measuring 6 to 8 μ long, straight or somewhat curved. The bacteria divide by binary fission. The cells occupied by the microörganisms do not contain any fat or urates and the nucleus is always normal.

The cockroaches present the bacteriocyte condition; there are no mycetomes nor complexes of mycetocytes nor, more strictly speaking in this case, bacteriocytes.

In *Blattela germanica* several rows of bacteriocytes are found in the fat body. The youngest eggs in the oviduct are free from infection. Somewhat older eggs, however, show several organisms on their surface. With the growth of the egg the number increases so much that later several layers surround the periphery. The direct wandering of the bacteria out of the host cells through the follicular membranes into the eggs, has not been observed, but since the fat body surrounds the ovarioles, this is highly probable.

In *Periplaneta* bacteriocytes have been found in the oviduct. No bacteria have been noticed at the periphery of young oöcytes, but on observing large ones a number can be seen on the surface that have wandered out of the bacteriocytes, and must have made their way out through the follicular membrane. As the egg grows the bacteria become much more plentiful on the surface forming a closed layer around the entire egg. Soon the microorganisms become more concentrated at both poles and many are seen to lie perpendicular to the egg. In freshly laid eggs the bacteria are found within under the vitelline membrane, so they must have left the follicle, but the exact method has not been traced.

In Blattids generally, the later development takes place as follows: The organisms are concentrated in the middle of the yolk. The vitellophags also become infected. When the germ band grows around the yolk the bacteria get into the embryonic intestinal lumen. After a time they leave the lumen, wander through the intestinal epithelium and get into certain fat cells which they modify as previously shown. They take possession of the adipocytes and render them functionless as fat cells.

While the morphological details are not complete, they are very interesting. I found very similar conditions in the two species with which I worked, namely, *Periplaneta americana* and *Parcoblatta virginica.*

One of the most striking things in connection with this work is the definite wanderings of the microörganisms. They are motile forms in the insects studied by me, but that would not account for the prescribed course which they follow. I believe one must postulate a chemotropic response between the bacteria and certain host tissues in order to account for such behavior.

2. The Morphological, Cultural and Biochemical Characters of the Organisms Cultivated from Parcoblatta virginica and Periplaneta americana.

By dissecting cockroaches that portion of the fat body in which the bacteriocytes are embedded can be easily removed. It is best to sacrifice one or two animals by exploration dissections, and by staining the excised tissue, in order to obtain an idea of the exact position of the desired cells and their microörganisms. When the region of the bacteriocytes has been discovered a fresh animal can be taken, etherized, pinned to a paraffin tray ventral side up, washed off with some sterilizing agent such as alcohol or a mixture of alcohol and corrosive sublimate and dissected with sterile instruments. The excised bacteriocytes with their contents can then be directly placed in the different sterile media and incubated.

The following are descriptions of the morphological, cultural and biochemical characters of the two species of organisms; one from *Parcoblatta virginica* and the other from *Periplaneta americana*. I consider it rather useless to give these bacteria specific names. To begin giving names to all of the thousands of symbionts, I think, would confuse rather than assist matters. It would be far better accurately to designate the host and then simply to refer to the genus of microörganism. In the case of disymbiotic and trisymbiotic insects one can refer to the two or three genera of symbionts associated with the host. If the two or three symbionts all belong to the same genus, the latter can be designated and the organisms labelled *a, b, c,* or 1, 2, 3.

The two species of organisms investigated by me are rather pleomorphic in cultures. Some individuals are straight like a bacillus; others are comma- or crescent-shaped like a spirillum. In the host cells the individuals are nearly all of the spirillum form. For this reason and owing to the fact that the organisms have one polar flagellum and do not form endospores, I am placing them in the genus *Spirillum*.

The organisms found by me in *Parcoblatta* and *Periplaneta* seem to differ from the morphological description of *Bacillus cuenoti* of Mercier. Since the cultural and biochemical characters of *B. cuenoti* were not described it has been rather difficult to make a comparison, however. The organism of Mercier seems to be a true bacillus, forming endospores of oval shape. The two organisms which I cultivated form no endospores.

Spirillum from Parcoblatta virginica.

Morphology.—1.5 per cent. neutral, nutrient agar, short and long rods. In nutrient bouillon longer rods, some curved, others straight. Average length 1.5–2 μ. Stains readily. Gram negative. No endospores. Motile. A single flagellum attached to one of the poles.

Nutrient Agar Stroke.—1.5 per cent. neutral. Growth: abundant, filiform, flat, glistening, smooth, opaque, butyrous. Odor absent. Medium greened.

Nutrient Bouillon.—Ring, clouding strong, odor absent, sediment abundant.

Gelatin Stab.—Growth best at top; line of puncture filiform. Liquefaction begins in two days, crateriform. Color of medium unchanged.

Litmus Milk.—Reaction not changed. Not coagulated. Reduction complete in eight days.

Milk.—Clearing without coagulation. Medium and consistency unchanged. No peptonization.

Potato.—Growth abundant, spreading, flat, glistening, smooth, butyrous. Odor absent. Medium unchanged.

Nutrient Agar Colonies.—1.5 per cent. neutral. Growth rapid at 35° C.; round, smooth, slightly convex, edge entire, amorphous. Diameter 1–1¾ mm.

Nutrient Gelatin Colonies.—Growth rapid, irregular, smooth, crateriform, edge undulate, liquefaction saucer.

Indol test negative.

NH_3 test faintly positive.

Nitrates not reduced to nitrites.

Diastase Test.—Starch converted to sugar.

Strong catalase reaction in bouillon culture.

Faint peroxidase reaction with gum guaiac and H_2O_2 in sugar bouillon culture.

FERMENTATION OF CARBOHYDRATES WITH FORMATION OF ACID AND GAS.

	Gas.	Acid.
Dextrose	0	0
Levulose	0	0
Saccharose	0	0
Maltose	0	0
Lactose	0	0
Mannit	0	0

Spirillum from Periplaneta americana.

Morphology.—1.5 per cent. neutral nutrient agar, short and long rods. Some curved, others straight. In nutrient bouillon, some straight rods and many curved. Average length 1.5–2 μ. Stains readily. Gram negative. No endospores. Motile. A single flagellum attached to one of the poles.

Nutrient Agar Stroke.—1.5 per cent. neutral. Growth abundant, filiform, flat, glistening, smooth, opaque, butyrus, odor absent, medium unchanged.

Nutrient Bouillon.—Ring, pellicle, clouding strong, odor absent, sediment abundant and flocculent.

Gelatin Stab.—Growth best at top, line of puncture filiform, liquefaction begins in two days, crateriform. Medium unchanged.

Litmus Milk.—No acid, coagulation prompt. Prompt reduction.

Milk.—Coagulation prompt, extrusion of whey. Consistency unchanged. Medium slightly greened. No peptonization.

Potato.—Growth scanty, slightly spreading, flat, dull, smooth, butyrous, odor absent, medium unchanged.

Nutrient Agar Colonies.—1.5 per cent. neutral. Growth rapid at 35° C., round, smooth, flat, edge entire, amorphous. Diameter ¾–2 mm.

Nutrient Gelatin Colonies.—Growth slow, colonies minute. Round, smooth, flat, edge entire, liquefaction saucer.

Indol test negative.

NH_3 *test* positive.

Nitrates not reduced to nitrites.

Diastase Test.—Starch converted to sugar.

Strong catalase reaction in bouillon cultures.

No peroxidase reaction.

FERMENTATION OF CARBOHYDRATES WITH FORMATION OF ACID AND GAS.

	Gas.	Acid.
Dextrose	0	0
Levulose	0	0
Saccharose	0	0
Maltose	0	0
Lactose	0	0
Mannit	0	0

It will be noticed from the foregoing descriptions that the morphological characters of the two species of spirilla are almost identical and if investigation were carried no further, one would doubtless consider the organisms from the two species of cockroaches as identical. Only in the cultural characters do the differences become striking and therefore I believe I am justified in considering the microörganisms as two distinct species. Taking Mercier's meager description of the symbiont from *Blatella* into consideration we should be inclined to assume that every living species of cockroach harbors a separate species of microorganism.

I did not test the pathogenicity of the two cultures on mammals. Perhaps this should have been done, since at present a great amount of interest centers on disease transmission by insects. Cockroaches are known to carry about and disseminate bacteria mechanically on their feet and in their intestines. Nevertheless, investigators interested in this matter might do well also to consider the intracellular organisms. Cockroaches are also in reality living reservoirs of thousands of bacteria enclosed within certain of the hosts' cells.

3. The Biochemical Activities of the Intracellular Organisms Studied.

First of all from the sugar tests it is interesting to note that the intracellular organisms do not ferment any of the sugars used. This is very fortunate for the insects. Undoubtedly sugar is present in some form taken in as food and if fermented by zymase secreted by the symbionts might anesthetize the animals with alcohol and CO_2.

The inverting enzyme sucrase is not produced by the *Parcoblatta* symbiont, but the *Periplaneta* organism inverts saccharose to levulose and dextrose.

Since the organisms are present in the fat body of adults and in the yolk of eggs, it seemed possible that they might have something to do with the metabolism of fat. Experiments with ethyl butyrate, milk fat and other fats failed to demonstrate the existence of lipase.

The cultural features demonstrated the fact that gelatin was liquefied. In other words it was evident that the organisms secrete a protease. All proteins, however, are not hydrolyzed. For instance, casein does not seem to be effected for after the organisms have been grown in milk for the prescribed time the biuret test did not show the presence of propeptones or peptones.

It was found that both species of intracellular organisms produce diastase. The organisms were grown in sugar-free bouillon for ten days. After that time the cultures were poured into some pure potato starch paste with 2 per cent. thymol and incubated for 48 hours. Thereupon Fehling's solution was reduced showing that sugar had been formed. Check tests accompanied the experimental tests.

The tests for oxidizing enzymes really do not signify much, for they can be obtained with cultures of almost any bacterium such as *B. coli communis* or *B. prodigiosus.* However, both symbionts produce catalase and the *Parcoblatta* organism demonstrated the presence of peroxidase.

We may summarize the enzyme activities on the following table and then attempt to correlate some of them with the metabolism of the insects.

ENZYMES PRODUCED BY THE INTRACELLULAR ORGANISMS.

Symbionts.	Zymase.	Invertase.	Diastase.	Protease.	Lipase.	Catalase.	Peroxidase.
Parcoblatta organism	0	0	+	+	0	+	+
Periplaneta organism	0	+	+	+	0	+	0

It will be seen from the preceding table that the enzymes common to both species of symbionts are diastase, protease and catalase. From the standpoint of Blattid metabolism, I think, diastase and protease important. I was disappointed in not being able to demonstrate the existence of lipase, for conditions generally made me feel sure that there was an association between the microörganisms and fat metabolism.

Both species of insects studied feed primarily on carbohydrates (starches) and proteins, so it was encouraging to be able to demonstrate the two enzymes that effect these two classes of foods. Of course, the insects may feed on fats to some extent, but I think the statement that carbohydrates and proteins constitute their principal diet is fairly safe.

Philiptschenko (1907) studied the physiology of the fat body in *Blattela*. He did not mention the intracellular organisms, but found glycogen, proteins, fats and urates stored in great quantities especially in young animals.

From all of these facts, it is easy to assume that the symbionts assist in the carbohydrate and protein metabolism through enzyme secretion. Of course, the catalase may serve an oxidizing purpose, but I do not attach much weight to such a statement for the reason that protoplasm generally will give this as well as the peroxidase reaction in most cases. Nor do I lay any emphasis on the invertase found in the *Periplaneta* organism. It may serve a purpose, but this is not evident.

4. Conclusions.

In speaking of the intracellular organisms of insects, the word symbiont has been used rather freely in the literature. I have also made use of the word more for the sake of convenience than because I am convinced that we are confronted with true symbiosis. From the morphological studies presented by others, and from my own work it is a great temptation to refrain from thinking of a symbiotic relation. From my experiments it is easy to assume that the insect derives some benefit from the organisms as digestive organs, but what possible benefit can the microorganisms derive? Buchner's suggestions that the intracellular organisms are benefited by being protected within the insects from the drastic atmospheric influences of heat, cold, desiccation, etc., are a little far-fetched. Nor does Buchner's view that the organisms are also assured constant nourishment and opportunity to propagate appeal to me. Millions of bacteria and other organisms not found in the cells of animals manage to live and propagate just as well if not better. It is also necessary to remember that the insect tissue or serum must produce some powerful inhibitor in order to hold the propagation of the microörganisms within a reasonable limit, otherwise the host would be killed. The presence of an inhibitor is surely a handicap in the struggle for existence.

I think the interesting relations discussed had their origin in true parasitism, possibly, in disease. During an early period the organisms now found as harmless entities within the insects were parasites producing pathologic conditions and disease. It seems to me, that such structures as the mycetocytes, bacteriocytes and mycetomes are a survival of previous profound pathologic changes. Later acquired immunity principles became heritable and the toxins and other effects came under control. The insects could not fully rid themselves of the invaders on account of the fact that transmission from generation to generation had established itself with unerring precision. Still later, the microorganisms lost all of their harmful effects and since they secreted a number of enzymes that proved serviceable to the hosts, the latter were not exterminated, but were probably diverted from their normal phylogenetic course. It is interesting to reflect what would have happened to those insects that harbor intracellular organisms if they had never been invaded by the parasites. If the hosts had not been able to overcome the infection they would have become extinct or at least reduced numerically to an appreciable extent. However, the insects were able to adapt themselves to profound changes and the phylogenetic history must have likewise taken another course. It is impossible to imagine that such structures as the pseudovitellus, mycetocytes and mycetomes with their inhabitants could have developed without altering the physiology and morphology and consequently the habits of the host.

The microörganisms may, as Buchner suggests, derive some benefit from the association, but this is not clear since they probably derived much more benefit as true parasites or disease producers. Judging from the ease with which the Blattid symbionts can be cultivated on almost anything, I feel sure that had they succeeded in exterminating all Blattids, they would still persist today living possibly as saprophytes under much more advantageous conditions.

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BIOLOGICAL BULLETIN

SOME CONSIDERATIONS CONCERNING THE NATURE AND ORIGIN OF PHYSIOLOGICAL GRADIENTS.

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Author's Abstract.

The paper is a survey of the evidence bearing upon certain aspects of the problem of integration or pattern in organisms. The organism represents a definite pattern or integration of some kind. The pattern of the organism is of a higher order of magnitude than protoplasmic pattern since it involves masses of protoplasm or even cells each of which may possess the entire protoplasmic or cell pattern. Protoplasm apart from environment does not appear to possess any inherent mechanism for originating such pattern.

While specific differences appear in the development and differentiation of pattern in each organism, in its more general features such as polarity and symmetry, this pattern appears to be largely independent of specific differences in protoplasmic constitution. This fact suggests that it represents primarily a non-specific or quantitative condition or relation in a specific protoplasm.

Many different lines of evidence which are briefly reviewed indicate, first, that the simplest form of axiate pattern is primarily a gradient in physiological condition, involving the fundamental metabolic reactions as well as various other factors, and second, that such gradients arise in the final analysis from differential exposure to external factors which affect the rate of protoplasmic activity. The primary physiological relations established in this way are those of excitation and transmission but from the first moment of differentiation chemical or transportative correlation becomes possible and plays an increasingly important rôle in the progress of development.

The living organism is manifestly not simply a fortuitous aggregation of substances but represents an order or unity, an integration of some sort, and the problem of the nature of this order and unity, the problem of organismic integration[1] is one of the fundamental problems of biology. The organism consists in each particular case of a certain material, a protoplasm possessing a specific inherited constitution and it represents a certain pattern which is in some way established in this material, and the problem of organismic integration includes both the problem of pattern and the problem of material. Pattern in the sense in which the word is used here is not merely nor primarily the visible morphological order and arrangement of parts or organs, but rather the underlying physiological order and relation of which the visible morphological order is one result, and the problem of pattern is the problem of the nature of this order. But since organismic pattern always appears in the material which we call protoplasm it is evident that the problem of material, *i.e.,* the problem of the physico-chemical constitution of each particular protoplasm in which the pattern appears is also of fundamental importance. Obviously organismic pattern must be of a kind which is possible in the protoplasm in which it appears and to this extent pattern must depend upon and be determined by the nature of the material. Moreover, protoplasm itself possesses a pattern, that is to say the problem of material resolves itself again into problems of pattern and material, the material in this case consisting of the various constituents of protoplasm, colloid particles, lipoids, electrolytes, water, etc., and the pattern of the order and the relation of these constituents. Similarly for each constituent of a protoplasm the two problems exist and so on until we attain the fundamental conceptions underlying physics and chemistry.

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[1] In view of the fact that the word "organism" which implies the existence of a unity and order in the entity so designated is universally accepted and employed, the word "organismic" is not only biologically and etymologically justified, but fills a need which is becoming more and more apparent.

At present, however, we are primarily concerned with the question of the organismic order or pattern as it appears in the protoplasmic material and we must accept protoplasm with its physico-chemical constitution and pattern as given, and attempt to determine how the organism differs from and arises out of protoplasm.

Organismic pattern appears in general to be of a different order of magnitude from protoplasmic pattern. It involves the appearance of orderly differences in and relations between regions or masses of protoplasm or cells each of which may possess the entire protoplasmic pattern. This fact alone presents great difficulties to any preformistic conception of organismic pattern, for protoplasmic pattern alone does not appear to afford any basis for the origin of this pattern of a much higher order of magnitude. Apparently organismic pattern is in some way superimposed on protoplasm and unless we are content to accept the "vitalistic" conclusions as a solution of the problem, we are forced to believe that in the final analysis organismic pattern arises in some way through the relations between protoplasm and the external world.

Second, organismic pattern as regards its more general features is apparently to a large extent independent of the specific differences in constitution of the different protoplasms. This independence is clearly indicated by the fact that only three types of spatial order appear in organismic pattern. These three are the surface-interior, or centered pattern in which the order is spatially referable to a point, the axiate pattern, in which the order is referable to a line, the axis, and the bilateral pattern, in which the order is referable to a plane passed through the line representing the axis. The spatial plan or pattern of all organisms represents either some one of these patterns or some modification or combination of one or more of them. If organismic pattern were inherent in protoplasm and therefore determined entirely by its specific hereditary constitution we should expect a far greater diversity in the general spatial order in organisms. The diversity appears, however, not in the general pattern but in the character and course of differentiation of its parts, and the degree and character of physiological relation between them. The liverwort *Marchantia* and the flatworm *Planaria* are both axiate and bilaterally symmetrical, *i.e.*, the general spatial pattern is essentially identical in both, but they are very different organisms because the materials, the protoplasms, in which the pattern exists are very different in constitution. Likewise different species of liverwort and different species of planarians differ in definite and characteristic ways because, even though the general features of the liverwort pattern or the planarian pattern are the same in all, the pattern is so to speak worked out or developed in specifically different protoplasms. And finally, both in individual development and in evolution we find in general a progressive complication in the details of organismic pattern, even though the same general features may exist in widely different stages of development and in widely different organisms, and the course of this complication differs both in the development of different species and in different lines of evolution.

These facts as well as many others suggest that the primary features of organismic pattern consist in a non-specific or quantitative order arising in some way in a protoplasm of specific constitution. We must then, in attacking the problem of organismic pattern, attempt first of all to determine whether there is any evidence for the existence of such a quantitative order as the basis of organismic pattern, and, if we find such evidence, how such an order originates in protoplasm. If the conclusion stated above is correct, viz., that organismic pattern in the final analysis must be determined, not by protoplasm alone, but by the relations between protoplasm and its environment, the problem of the origin of organismic pattern is the problem of determining what particular relations give rise to the primary features of this pattern and how. Two questions then are before us: what is the nature of organismic pattern in its simplest or most primitive stages or forms, and how does such pattern originate?

The Simplest Stages of Organismic Pattern.

Surface-interior Pattern.—The cell is primarily an organism, though it may be integrated with other cells to form a part of a multicellular organism. While we have no positive knowledge concerning the origin of cell pattern, the structure of the cell in general suggests that it is primarily what we may call a surface-interior pattern. If this is true the differentiation of nucleus and cytoplasm from the primitive protoplasm possessing in some degree the functions of both, must have resulted in the first instance from differences and relations between surface and interior. Some organisms appear to be even simpler in pattern than the ordinary cell, but most of them are so minute and show so little differentiation of parts that our knowledge of their pattern is very fragmentary. Even in the simplest organisms, however, we should expect to find at least a surface-interior pattern. In the absence of any positive data, discussion of the origin of cell pattern and of still simpler patterns is of value only in the light of what we can learn concerning other organismic patterns and is therefore postponed (see pp. 176–178).

The surface-interior pattern is primarily a completely radial, spherically symmetrical, or centered pattern and, assuming that organismic pattern arises through relation between protoplasm and environment, this pattern represents such relation in its most general and primitive form. While some cells and some still simpler organisms may possess this pattern alone it is certain that most organisms including all multicellular forms, at least many unicellular forms and probably some of those simpler than cells, possess not only a surface-interior pattern but also an axiate or polar pattern and primarily either a radial or bilateral symmetry with asymmetric modifications in various cases. Both the course of organismic evolution from very early stages, as well as the development of the individual, are based upon this axiate pattern. I have already pointed out (pp. 149–150) that the general similarities of organismic pattern in different protoplasms suggest that such pattern is, at least as regards its fundamental features, in large measure independent of the differences in the specific constitution of different protoplasms and therefore essentially quantitative, *i.e.*, it apparently represents a quantitative relation in a specific protoplasm. It now becomes necessary to examine the data of observation and experiment with reference to the question of the nature of the axiate pattern.

Axiate Pattern.—Many different lines of evidence agree in indicating that axiate pattern in its simplest form is essentially quantitative in character and consists in graded differences in the rate of the fundamental dynamic activities of protoplasm and in the conditions associated with these activities. These graded differences in physiological state have been called axial gradients because so far as the evidence goes they are the primary indications of the existence of an axiate pattern (Child, '15*c*). They have also been called metabolic gradients, not because it is assumed that they are purely or primarily metabolic in character, but because our knowledge of protoplasm in general indicates that metabolism and more specifically oxidation is a fundamental factor in life and the chief source of the energy of living organisms and because the data of observation and experiment indicate that differences in the rate of metabolism and particularly of oxidation are characteristic features of these gradients. Protoplasm is a system in which the chemical reactions of metabolism are so intimately associated with other factors, *e.g.*, colloid dispersion, active mass of enzymes, permeability of limiting surfaces, electrolyte and water content, etc., that to attempt to distinguish one particular factor rather than another as primary is at present impossible. The axial gradients are not simply oxidative or metabolic gradients but gradients in general physiological state of the particular protoplasm concerned, and in this physiological state oxidation and associated with it other metabolic reactions are important factors. The term "metabolic gradient" as used in this connection means only that the metabolic factor is a characteristic feature of the gradients. The term "physiological gradient" which avoids all specific implications might be substituted for it.

In the development and differentiation of the axiate pattern the apical region or head of the organism arises from the region of greatest acitivity or highest metabolic or oxidative rate—the "high end" of the major or polar gradient—and other organs at different levels of the gradient. The simplest forms of radial symmetry in which there is no differentiation between different radii are in reality nothing more than an apico-basal pattern and only an apico-basal gradient is present. In the more complex forms of radial symmetry a number of radial axes or gradients arise and the radially arranged parts differentiate in relation to these. In bilateral patterns there is not only a gradient along the longitudinal or polar axis, but bilateral gradients are also present. In most bilateral invertebrates the high region of these bilateral gradients is apparently represented by the median ventral region and in the vertebrates by the median dorsal region. The evidence indicates that the chief axial gradients appear primarily in the superficial regions of the cell or multicellular body and in many of the simpler organisms, *e.g.*, many protozoa (Child, '14*b*) and plant cells they are throughout life present only or chiefly in the superficial regions, but where definite localized internal organs with an axiate pattern exist, these also show axial gradients in all cases examined, though these organ gradients do not necessarily coincide in direction with the primary gradients. As was pointed out nearly five years ago (Child, '15*c*, pp. 54, 60) the primary gradient or gradients may persist throughout life, but do not necessarily do so. They may undergo modification and complication in various ways during the course of development: the originally quantitative relations may become qualitative, new gradients may arise in certain parts or organs, in some cases preëxisting gradients may be broken up, obliterated or reversed, one kind of axiate pattern may be replaced by another, and so on. In all cases, however, the sequence of events is definite and orderly and many features of it can already be interpreted in physiological terms, and usually the pattern even in the fully developed organism shows a very definite relation to the primary gradients. The evidence for the existence of metabolic or physiological axial gradients is varied and extensive and only a brief summary is possible here.

Structural and Developmental Gradients.—First of all many eggs and embryos show an apico-basal gradient in protoplasmic structure and content, *e.g.*, the gradient in yolk accumulation in many animal eggs and embryos and the gradient in protoplasmic density and vacuolation in many plant embryos (Child, '15*c*, Figs. 8, 19) and in the vegetative axes of many of the simpler plants.

A gradient in the rate of cell division, growth and differentiation in relation first of all to the primary or apico-basal axis, later in relation to other axes, is a very general feature of at least the earlier stages of development. This gradient appears first in the rate of cell division and size of cells along the apico-basal axis in a large proportion of both animal and plant eggs (Child, '15*c*, Figs. 10, 11, 18, 19) and in many plant axes, as a gradient in rate of division and cell size from the growing tip basipetally (Child, '15*c*, Figs. 20, 21, 22, 36–39). It also appears in the progress of morphogenesis and differentiation along the axes particularly in animals, although in plants, except for the fact that the growing tip itself remains embryonic, the course of differentiation is also in general basipetal. Moreover, in bilateral animals and plants a similar bilateral developmental gradient appears more or less clearly. In at least most bilateral invertebrates differentiation of the body-wall and its organs progresses from the median ventral region laterally and dorsally (Child, '15*c*, Fig. 13) and in vertebrates from the median dorsal region laterally and ventrally, as a moment's examination of the chick embryo shows. The so-called law of antero-posterior development is merely a recognition of the existence of this developmental gradient in the longitudinal axis, and it is, moreover, only a partial statement of what might be called the law of axiate development. We also find evidences of the existence of a polar axial gradient in the rate of regulatory development and the position and proportions of organs in isolated pieces from different levels of the body in various forms (Child, '07*b*, '07*c*, '11*b*; Hyman, '16).

The Evidence from Susceptibility.—The existence of the axial gradients is perhaps most readily demonstrated through the susceptibility of organisms to the action of various external agents. It has been determined experimentally for many species of animals including all the chief phyla and many of the smaller groups and nearly a hundred species of algæ among plants that axial gradients in susceptibility to the action of at least a wide range of external agents exists. The agents used in these experiments include cyanides, many anesthetics such as alcohol, ether, chloroform, chloretone, some of the urethanes, etc., carbon dioxide and various acids, alkalies, neutral salts, certain alkaloids, vital dyes and physical conditions such as extremes of temperature and the negative factor lack of oxygen.[1] Potassium cyanide has been much used in these experiments first because very low concentrations are effective and second, because it has been found by a large number of investigators to be a powerful inhibitor of protoplasmic oxidation, and susceptibility to cyanide may therefore be regarded as to some extent a comparative measure or indicator of rate of oxidation in the parts, organs or individuals of a particular species.[1]

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[1] The data on susceptibility as determined by death and disintegration of cells or tissues, so far as they have been published, appear in the following papers: algæ, Child, '16*c*, *e*, '17*a*, *b*, '20*a*, protozoa, Child, '14*b*, Hyman, '17; cœlenterates, Child, '18, '19*b*, Child and Hyman, '19; ctenophore, Child, '17*c*; Planaria, Child, '12, '13*a*, '13*b*, '14*c*, *d*, '16*b*, '19*c*, *d*; echinoderm eggs and embryos, Child, '15*a*, '16*a*; annelids, Child, '17*d*, Hyman, '16; amphibia, Bellamy, '19; miscellaneous, Child, '14*a*, '15*c*, pp. 50–62. Further data on susceptibility gradients in protozoa, ctenophores, hydrozoa, flatworms, echinoderms, annelids, fishes and amphibia obtained by Bellamy, J. W. MacArthur, Hyman and Child are not yet published.

Comparison of the data on susceptibility with data obtained by other methods has shown that a general non-specific relation between susceptibility and physiological condition exists, at least in the simple organisms and early developmental stages, where the differentiation of organs is not so far advanced that a more or less specific action of particular agents or particular organs is involved. This relation is as follows: to a certain range of concentrations or intensities of the agents used, which is experimentally determined to be above the limit of acclimation or tolerance for the particular species examined, susceptibility varies directly with, though not necessarily proportional to the general physiological activity of the protoplasm. To a certain lower range of concentrations or intensities, also experimentally determined for each species, the rate and degree of acclimation or acquirement of tolerance vary directly, though not necessarily proportionally, to the general physiological activity of the protoplasm, and the rate and degree of recovery after temporary exposure to the action of the agent also vary in the same way. The metabolic reactions and particularly the oxidations are important factors in the general physiological activity of protoplasm and many lines of evidence show that susceptibility may be used within certain limits and with certain precautions as a rough comparative measure or indicator of the rate of oxidation. This of course does not mean that all the agents used in determining susceptibility act directly on the oxidations. Undoubtedly different agents act in very different ways upon living protoplasm but the general non-specific character of the susceptibility gradients indicates the interrelation of different processes and conditions in the protoplasmic system. The difference between a region of high and one of low rate of oxidation unquestionably involves not simply the oxidative reactions but many other factors, *e.g.*, colloid dispersion, permeability of limiting surfaces, active mass of enzymes, etc., and within physiological limits change in the physiological state involves changes in all these factors. Apparently the general relation between susceptibility and physiological state is primarily an expression of the fact that the dynamic equilibrium of the more active state is, on the one hand, more readily upset by any extreme external action than that of the less active state and, on the other, undergoes more rapid and more complete recovery from temporary alteration. In short, so far as susceptibility is non-specific and quantitative, it is apparently an indicator of the quantitative aspects of physiological state in protoplasm. Susceptibility to the higher, directly lethal concentrations and intensities can be determined by survival time and in many cases loss of motor activity, swelling or shrinkage of cells or other changes preceding death can be used as a check on survival time. To lower concentrations, which are not directly lethal, susceptibility can be determined by the degree of inhibition, acclimation or recovery in growth, development, motor activity, etc., in different body-regions or individuals.

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[1] For the literature on the action of cyanide see Hyman, '19. Allen, '19*a*, and Hyman, '19, have shown that KNC greatly decreases oxygen consumption in *Planaria*. I have found that KNC decreases CO_2 production in *Planaria*, though apparently less than it decreases oxygen consumption and that KNC and lack of oxygen are to some extent additive in their action on CO_2 production (Child, '19*c*). Lund claims that KNC does not decrease oxygen consumption in *Paramœcium* (Lund, '18), but it may be pointed out that the concentrations of KNC in his experiments give a solution of very high alkalinity which produces extreme stimulation with intense motor activity often continuing for several hours until the inhibiting action of the cyanide appears. With cyanide solutions brought almost or quite to neutrality there is no apparent stimulation and no increase in motor activity. Lund did not neutralize his KNC solutions, therefore the total oxygen consumption undoubtedly does not represent the action of cyanide alone, but includes the stimulating effect of the alkali. Repetition of the experiments in cyanide solutions with H ion concentration approximately that of the normal environment of *Paramœcium* is of course necessary before final conclusions are possible, and a repetition will be undertaken in this laboratory as soon as opportunity permits.

More recently Lund ('20) has shown that KNC and lack of oxygen do not act in the same way upon *Planaria* since with lack of oxygen CO_2 production is not decreased during the period of the experiment, while it is decreased by KNC. The method of CO_2 determination used by Lund is, as he has shown (Lund, '19), not very accurate, but leaving out of account the possibility of technical error, the work of various investigators indicates very clearly that the effect of cyanides on protoplasm is not identical with the effect of lack of oxygen. I have pointed out that cyanide and lack of oxygen are to some extent additive in their action and that their action must therefore be similar or identical in certain respects. Up to the present, therefore, Lund's work affords no grounds for modifying the conclusions reached by earlier investigators concerning the action of cyanide, and we are still justified in believing that susceptibility to cyanides is in a general way and to some extent a measure or an indicator of rate of oxidation.

The data on susceptibility have demonstrated the existence in a large number of organisms of definite susceptibility gradients as characteristic and as the earliest distinguisable features of axiate pattern, not only for the organism as a whole, but for various axiate organs and parts. In all cases the differences in susceptibility correspond to differences in structure, rate of growth, development and differentiation, and in certain cases it has been possible to show that susceptibility gradients correspond to gradients in oxygen consumption and carbon dioxide production.

In various cases it has been possible to follow the apico-basal or antero-posterior gradient from the egg and to show the modifications which occur during the course of development, and the appearance of new gradients in particular organs, or in connection with agamic reproductive processes. In the simpler organisms the susceptibility gradients are wholly or largely superficial. In the ciliate infusoria, for example, axiation and morphological differentiation are, at least to a very large extent, confined to the ectoplasm, and in the forms which have been examined the ectoplasm alone shows a gradient in susceptibility (Child, '14*b*). As regards many plant cells the same is also true. In many of the monosiphonous algæ with elongated cells the apico-basal susceptibility gradient is very distinct within the limits of a single cell (Child, '16*c, e,* '17*a, b,* '20*a*) as well as in the axis as a whole. Modifications with advancing age, cessation of growth, budding, etc., appear very clearly in the changes in susceptibility.

In some animals the primary gradients persist throughout life, while in others the original gradients may completely disappear during development. In the development of the hydroid, for example, a very distinct gradient appears in the unfertilized egg and cleavage stages, and the planula shows a well marked apico-basal gradient. But as the time of attachment of the planula approaches, this gradient gradually becomes less and less distinct, and the planula attaches itself by the end which was originally apical, a fact which has long been known. After attachment the original gradient disappears completely, a new gradient in the opposite direction arises at the opposite end, and the first hydranth arises from the high end of this new gradient. In other words, the phenomenon which Loeb and others have called heteromorphosis is a normal feature of hydroid development. Again in the polyclad turbellaria the early stages of development show a very distinct antero-posterior susceptibility gradient, the head arising from the most susceptible region, but in the later stages, so far as they have been examined, a reversal in the susceptibility relations occurs, at least in the superficial regions, and in the adult worms the ectoderm of the head is less susceptible than more posterior levels. These data on hydroids and polyclads have not yet been published in full. In the annelids a second gradient in the opposite direction from the primary antero-posterior gradient appears at least superficially in the posterior regions as the result of the development of the posterior growing region (Hyman, '16; Child, '17*d*). But whatever the changes, they are definite and orderly and associated with the course of development in each species.

As development and differentiation progress, the indications of more or less specific relations between particular regions or organs and particular agents become more frequent. In *Planaria dorotocephala,* for example, we find that the lateral margins of the body are more susceptible superficially than the median regions to alkalies, while in neutral and acid agents there is little difference between median and lateral or the median ventral region is slightly more susceptible than dorsal and lateral. With methylene blue of certain concentrations Mr. McArthur has found that susceptibility of the median ventral region is distinctly greater than that of dorsal and lateral regions. The early embryonic stages of *Planaria* have not been available for work on susceptibility, but the data on other flatworms and various other bilateral invertebrates indicate that primarily the susceptibility of the median ventral region is higher than that of dorsal and lateral regions and the outgrowth of tissue from cut surfaces in pieces of adult *Planaria* suggests a greater parenchymal activity in the median ventral region. In the previously published data on the gradients in *Planaria* (Child, '13*b*) it was noted that the relations as regards median, lateral and dorsal were not entirely clear. As regards the alimentary tract, various facts indicate that in the adult the region of greatest activity and highest susceptibility is in the middle of the body about the base of the pharynx and that a gradient of decreasing activity extends in both directions from this region, but since the alimentary tract is an internal organ and is not readily separable from other parts, it is difficult to obtain conclusive evidence on this point. The antero-posterior gradient of ectoderm and body-wall persists throughout life, but with the appearance of new zooids at the posterior end new gradients arise in that region, or more strictly speaking, the original gradient undergoes modification. Apparently the primary embryonic relations have undergone more or less alteration, even in *Planaria.*

With the appearance of more or less specific relations between particular regions or organs and particular agents, the value of the susceptibility method as a means of distinguishing general quantitative differences and relations is of course greatly decreased. In the higher animals apparent specificity of relation between particular organs and particular agents is much more evident than in lower forms and becomes increasingly complex with the progress of differentiation, but even in these forms the general non-specific susceptibility relations appear in the earlier developmental stages, at least in all forms examined. While caution is always necessary in interpreting the data of susceptibility in non-specific quantitative terms, it has become more and more evident as the data have accumulated that general non-specific susceptibility relations do exist, particularly in the simpler organisms and in the earlier stages of development, and that they are indications of fundamental physiological features of organismic pattern.

It has also been possible to control and modify development in definite predictable ways through the differential susceptibility of different levels of the axial gradients. Such modifications consist in differential inhibition, differential acceleration, differential acclimation and differential recovery, each representing a definite teratological type. In cases of differential inhibition with respect to a gradient the degree of inhibition varies directly with the susceptibility and activity of different levels. The region of greatest activity is most inhibited and less active regions are less inhibited. In the apico-basal or antero-posterior axis for example, the degree of inhibition is greatest in the apical or head region and decreases basipetally or posteriorly, consequently the positions and proportions of parts are altered in a definite way, the apical or head-region being relatively smaller and the basal or posterior regions relatively larger than in the normal. Microcephaly, for example, is a characteristic result of differential inhibition along the polar axis. In differential acceleration the alterations of proportion are in the opposite direction and megacephalic forms result. In differential acclimation and recovery growth or development is first inhibited to some extent, but the more active levels of a gradient acclimate or recover more readily and more completely than the less active, so that in these cases growth or development is finally relatively more rapid or greater in amount apically or anteriorly than basally or posteriorly.

Similar modifications also appear with respect to the symmetry gradients. In differential inhibition in bilateral forms, for example, median regions are more inhibited, while in differential acceleration they are more accelerated than lateral, and in differential acclimation and recovery median regions are finally less inhibited than lateral.

These various modifications are, with respect to their more general features, non-specific as regards agents, and all except the differential accelerations which require the action of accelerating agents can be produced in some degree by a large number, probably by all agents which inhibit general protoplasmic activity and which in lower concentrations or intensities permit at least some degree of acclimation or recovery. Moreover, modifications of the same general type with respect to a particular axial gradient can be produced in widely different organisms, *e.g.*, flatworms, echinoderms, fishes, frogs. In many respects these definite developmental mdifications constitute the strongest evidence for the relation between susceptibility and the rate of fundamental activities of living protoplasm.[1] And finally, differential susceptibility, as a relation dependent primarily upon quantitative rather than upon specific or qualitative differences in the physiological state or activity of protoplasm, provides a simple and adequate basis for the interpretation of much of the work on experimental teratogeny and many of the teratological forms occurring as "accidents" in nature. The cases of cyclopia and microcephaly in fishes experimentally produced by Stockard ('07, '09, '10, '11, etc.) are essentially similar to the inhibited types of head in *Planaria* (Child, '11*a*, '15*c*, pp. 105–117) and to the differential inhibitions in the sea urchin (Child, '16*d*) and in amphibia (Bellamy, '19, and further data not yet published). All these cases involve a greater degree of inhibition of apical or anterior and median as compared with basal, posterior and lateral regions and all are non-specific in origin, *i.e.*, can be produced by the action of various agents and conditions.

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[1] Data on the control and modification of development through differential susceptibility have appeared as follows: Child, '11*a*, '16*d*, '17*d*; Bellamy, '19; and further data on hydrozoa, echinoderms and amphibia are still unpublished.

The susceptibility method makes no pretense of being an exact quantitative method of measuring metabolism or oxidation, nor is it to be regarded as taking the place of any other method of investigating physiological condition or rate of metabolism or oxidation. Its chief value is as a supplement to other methods. In the first place it enables us to demonstrate the existence of certain characteristic, non-specific, regional differences in physiological condition in organisms, which because of their unicellular character or their small size, or because of the complications introduced by separating different body regions are not available material for other more direct and more exact methods. Even in these forms, however, the action of external chemical agents is in general from the surface inward, consequently the information given by the susceptibility method concerns first of all the superficial regions of the cell or body, but it is possible in many cases to learn something concerning differential susceptibility of internal parts and organs.

Second, by the modification and control of development through differential susceptibility, the method enables us to show that the differences in condition indicated by differences in susceptibility are fundamental factors in organismic pattern. The conclusions concerning the relation between susceptibility and rate of metabolism or oxidation mean no more than that rate of metabolism or oxidation is so far as the evidence goes a factor in the conditions which determine susceptibility, but it is not claimed that this conclusion is universally valid. Undoubtedly in the more highly differential organisms and in more advanced stages of development the qualitative differences in different organs may determine differences in susceptibility which are more or less specific as regards both organ and agent, but in the simpler forms and the earlier stages the similarity of the susceptibility gradients in widely different organisms and with a great variety of agents renders their non-specific character sufficiently clear, and many lines of evidence, both direct and indirect, indicate their relation to rate of metabolism or oxidation. The nature and degree of that relation in each particular case and for each particular agent remains of course to be determined by other methods of investigation. The susceptibility method has served to bring to light certain characteristic features of organismic pattern which have not previously been clearly recognized, viz., the gradients, but conclusions concerning the exact nature of these gradients are possible only on the basis of all the different lines of evidence obtainable, and at present of course cannot be final.

Susceptibility in Relation to Permeability.—In many forms, both plants and animals, in which susceptibility gradients exist, corresponding gradients in the rate of penetration of certain substances, particularly the vital dyes, neutral red and methylene blue, which have been most extensively used in these experiments, have also been demonstrated.[1] The existence of these gradients in rate of penetration raises the question whether the susceptibility gradients are not primarily gradients in permeability of the protoplasmic surfaces to the agents used. While there is no doubt that a gradient in permeability is one aspect of the axial gradient, our conception of the relation between permeability and the gradient must depend very largely upon the terms in which we define permeability. If permeability is dependent only on the physical condition of the limiting surface and independent of chemical activity, the axial gradients are manifestly something more than mere permeability gradients. But the protoplasmic limiting surface or membrane is certainly polyphasic in constitution, since it is alive and the seat of more or less chemical activity, and its permeability depends upon its living condition and changes when it dies. Moreover, susceptibility as determined by the higher concentrations and intensities of external agents depends rather upon the destruction or alteration of the limiting surface as a living membrane than upon the passage of the agent through the living membrane into the interior of the cell. The susceptibility gradients can be demonstrated not only by agents to which the protoplasmic surface is highly permeable, *e.g.,* vital dyes, various anesthetics, but by those to which it is highly impermeable such as mercury and copper salts, and by extremes of temperature and the negative condition, lack of oxygen, which do not involve the action of any external chemical agent upon the surface.

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[1] Much of this work has been done by Mr. J. W. MacArthur and is not yet published. For some observations on algæ see Child, '20*a*.

A brief consideration of the question of permeability in relation to the action of KNC on *Planaria* is of interest here. The susceptibility gradients appear very clearly in KNC: in *m*/1000 KNC, for example, the survival time of the head of *Planaria dorotocephala* is about half or two thirds that of the least susceptible regions of the body-wall. It has been shown, however, that KNC produces a completely reversible decrease of 80–90 per cent. in the oxygen consumption of *Planaria* (Allen, '19*a*; Hyman, '19)., a fact which certainly indicates that the permeability of *Planaria* protoplasm to cyanide is relatively high, and that the differences in permeability in different regions cannot be very great. If the differences in survival time of different body-regions result from differences in rate of penetration of the cyanide, then the rate of penetration in the most susceptible regions must be at least nearly double that in the least susceptible regions, and if this is true, we should expect that in the determinations of oxygen consumption some parts of the body would be dead long before a total decrease of 80–90 per cent. had occurred. In fact, it is apparently not possible to interpret susceptibility to cyanide in *Planaria* solely in terms of rate of penetration.

Moreover, the phenomena of all differential acclimation and recovery in growth and development indicate very clearly that the metabolic activity of protoplasm is a factor in susceptibility. With the advance of our knowledge it becomes increasingly evident that the factors concerned in the permeability of living protoplasm are essentially those concerned in other aspects of life and that permeability is an expression of the physiological state of the plasma membranes. If we admit this, differences in permeability are themselves to some extent indicators of differences in physiological state, but it still remains true that susceptibility is not simply a matter of the rate of penetration of the plasma membranes but rather of the rate of killing or alteration of the membranes and superficial regions of protoplasm by an external agent. Different external agents may and undoubtedly do act chiefly or primarily upon different factors concerned in the maintenance of physiological state, but since these different factors are mutually associated in such maintenance, the general result as regards susceptibility, *i.e.,* the general effect on the physiological state, may be and is the same for at least many different agents. In short, susceptibility is within certain limits and in a general way an index of physiological state in protoplasm and the axial gradients in susceptibility are therefore significant, particularly when their existence is confirmed by other methods, as indicating the existence of non-specific or quantitative differences as the earliest distinguishable features of axiation.

Demonstration of the Gradients by Reduction of $KMnO_4$ and by the Indophenol Reaction.—The axial gradients have also been demonstrated in many forms as a differential in the rate and amount of reduction of potassium permanganate by protoplasm. It is a well known fact that $KMnO_4$ is reduced by protoplasm and the reduced salt appears on or in the protoplasm as a brown or blackish precipitate. All axiate forms examined including numerous protozoa, eggs, embryonic and larval stages or adults of the lower invertebrates, echinoderms and smaller arthropods and various algæ among plants show gradients in the rate of staining by permanganate corresponding to the gradients demonstrated by susceptibility and other methods. The precipitation of the reduced salt and the appearance of the brown color apparently begins on the external surface of the protoplasm, often within a few seconds after the organisms are brought into the solution and the differences in rate of precipitation and staining at different levels of an axis are usually very marked. Penetration into the protoplasm occurs rather slowly, its rate depending somewhat on concentration and it is certainly not to any large extent dependent on permeability of living membranes but rather on the killing of the protoplasm from the surface inward.

If the reaction is allowed to continue to completion in excess of permanganate the whole organism may become opaque black and no gradient is visible, but many small organisms thus stained, *e.g.,* blastulæ, hydroid planulæ, small monosiphonous algæ, can be made more or less transparent after such staining by hardening, clearing and mounting, and in such cases the gradient in staining appears. Under these conditions the gradient represents a gradient in the total amount of reduction of permanganate of which the protoplasm is capable and it is highly significant to find that the protoplasm of the apical region of a blastula or an alga axis, for example, is capable of reducing more permanganate than more basal levels. If the organism is first killed by some other agent, *e.g.,* various histological fixing agents, heat, etc., reduction and staining are uniform, or in some cases slight traces of the gradients are still present, but after a few days in alcohol they are completely absent in all cases examined.

Since the chemical reaction concerned here is an oxidation-reduction reaction, the rate and amount of reduction of permanganate must be associated in some way with the oxidative activity of protoplasm, the regions of higher rate of oxidation showing a higher rate and greater total amount of reduction. We find that the permanganate gradients correspond with those demonstrated by other methods, *i.e.,* the more active and more susceptible regions reduce permanganate more rapidly and in larger amount than the less active and less susceptible. This method is a very delicate one and, I believe, of considerable value as a means of determining regional and axial differences in physiological state, particularly in small organisms.

In certain cases the indophenol reaction has been used to demonstrate the existence of axial gradients in blastulæ and gastrulæ. This reaction is an oxidation which is catalyzed by oxidizing enzymes and which results in the formation of indophenol in the form of a blue precipitate. In living starfish blastulæ and gastrulæ (Child, '15*a*) a distinct apico-basal color gradient has been observed by this means, the formation of indophenol occurring most rapidly in the cells of the apical region. If the animals are killed by some other agent before the indophenol reaction the blue color is much less marked and the gradient is absent.

Gradients in Electric Potential.—Axial gradients in electric potential have been found by Drs. Bellamy and Hyman to exist in axiate animals so far as examined, the region of greatest physiological activity as indicated by other methods showing in general the highest electro-negativity through the galvanometer. These data are not yet published. Observations along these lines were first made by Mathews ('03) on hydroids, Waller ('03) on many different organisms and organs and Hyde ('04) on various eggs, and both Mathews and Waller point out the probable relation between electric potential and physiological or metabolic activity. Morgan and Dimon ('04) in a study of electric potential in the earthworm found that in general the two ends were electro-negative to middle regions and concluded that the potential differences were not related to physiological polarity. We know now, however, that the earthworm and other annelids develop very early a growing region of high physiological activity at the posterior end (Hyman, '16; Child, '17*d*) and that the body in later stages shows two gradients in opposite directions. The observations of Morgan and Dimon do not therefore conflict with those of others on other animals. More recently Tashiro ('17 and earlier papers) on the basis of his work on CO_2 production in the nerve fiber has pointed out the probable relations between the electric phenomena and metabolic activity in nerve, and Hyman ('18) has also suggested that bioelectric phenomena in general are primarily due to differences in metabolic activity. While differences in potential undoubtedly may arise in organisms from other causes than differences in metabolism or oxidation rate the facts in general indicate that such differences are very generally associated with differences in metabolic rate. In any case the axial gradients in potential indicate the existence of graded differences in physiological condition along the axes and in the light of other data, there can be little doubt that differences in rate of metabolism or more particularly oxidation play at least a very important part in determining the electrical gradients.

Oxygen Consumption and Carbon Dioxide Production in Relation to the Gradients.—The direct determination of respiratory activity in different body regions by means of oxygen consumption and CO_2 production requires the separation of the regions concerned and therefore introduces various complicating factors. Only in the simpler organisms is it possible to maintain such separated pieces of the body in anything like a normal condition, and even here the operative procedure involves stimulation and may be followed by depression, and cell division and growth begin at the cut surfaces within a few hours after the section. Moreover, in organisms with localized and differentiated internal organs the axial gradients do not necessarily run in the same direction in all organs and a piece of the body from a particular level may represent a low level of the body wall gradient and a high level as regards certain internal organs, or vice versa. In *Planaria* for example the mouth is near the middle of the body and the activity of the alimentary tract probably decreases from the mouth in both directions, but in the body wall the gradient is from the head posteriorly. If this is true, a piece of the *Planaria* body from levels near the head represents a high level of the body wall gradient and a low level of the alimentary tract, while a piece from near the mouth represents the reverse condition. Unless we can eliminate one or the other of these gradients the oxygen consumption and CO_2 production of such pieces is not likely to give us any very definite information as regards either gradient. In this case, however, we can bring the alimentary tract into a more or less quiescent condition by starvation, and then we find that a gradient in oxygen consumption and CO_2 production does exist in the body wall, the high end being at the head with a second rise in the region of the posterior zooid. The data along this line are as yet mostly unpublished, but one paper on CO_2 production in *Planaria* has already appeared (Robbins and Child, '20) and work on oxygen consumption has been done by Dr. Hyman with similar results. But the relative amount of alimentary tract tissue in pieces of a given weight taken near the head is less than in pieces taken near the mouth, so that even in animals which have been starved for some time the difference in total oxygen consumption or total CO_2 production of pieces from different regions is not strictly comparable with the regional differences in susceptibility of the body-wall. Where it has been possible thus far to use these methods we have found that the axial gradients demonstrated by other methods are also gradients in oxygen consumption and CO_2 production.[1]

Gradients in Organs.—By one method or another or by several different methods, the existence of physiological gradients in various axiate organs or parts of many organisms has been demonstrated, *e.g.,* in various reproductive axes and in the "hairs" of algæ (Child, '16*c, e,* '17*a,* '20*a*), in the larger, slow moving flagellum of *Noctiluca,* in the tentacles of hydrozoa

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[1] Allen ('20) has recently reported failure to find differences in CO_2 production corresponding to the antero-posterior gradient in pieces of *Planaria agilis.* This is a different species from the one used in this laboratory, but conditions are probably not fundamentally different in the two. It may be pointed out, however, that the differences in CO_2 production in pieces from different body-levels in *Planaria dorotocephala* are not very great (Robbins and Child, '20) and that Allen's failure to find such differences in *P. agilis* is probably due to the fact that the activity of the alimentary tract was not sufficiently decreased by starvation to permit the differences in the body wall to appear. A study of *P. agilis* is now being made in this laboratory by Dr. Hyman and it has been found that the alimentary tract of this species is considerably larger in relation to the size of the animal than in *P. dorotocephala.* An individual of *P. agilis* of given size is able to take in a much larger quantity of food than a *P. dorotocephala* of the same size. In well fed individuals of *P. agilis* oxygen consumption per unit of weight is only about two thirds that in well fed individuals of *P. dorotocephala* of the same size. Much if not all of this difference is undoubtedly due to the fact that a much larger proportion of the total weight of *P. agilis* than of *P. dorotocephala* consists of food in the alimentary tract or of reserves which are taking little or no part in respiration. Moreover, as might be expected, *P. agilis* starves and undergoes reduction much less rapidly than *P. dorotocephala* and Allen's failure to find an increase in rate of oxygen consumption in the later stages of starvation was due merely to the fact that the starvation period in his experiments was not long enough. Dr. Hyman has found that an increase in oxygen consumption does occur in this species as well as in *P. dorotocephala* and *P. maculata* in the later stages of starvation, but under similar experimental conditions the rise begins considerably later and progresses more slowly in *P. agilis* than in the other species.

(Child and Hyman, '19; Child, '19*b*) in the plate rows of Ctenophores (Child, '17*c*), the growing arms of echinoderm larvæ, branchiæ and sensory tentacles of various annelids, the growing tail of the ascidian and the amphibian tadpole, etc. Dr. Hyman has found that the embryonic heart of the chick and of the fish represents a susceptibility gradient with the high region at the sinus end. But the most extensive work on the metabolic gradient of any organ is that of Alvarez and his assistants on the vertebrate alimentary tract.[1] They have found in the small intestine a gradient in irritability, latent period, tone rhythm, conduction and susceptibility to various drugs and gradients in at least some of these conditions in the wall of the stomach and colon. Tashiro ('17 and earlier papers) has found a gradient in CO_2 production in certain nerves, the direction of functional conduction being down the gradient and in certain of these nerves a susceptibility gradient has been observed (Child, '14*a*).

In an investigation of the respiration of ground nervous tissue C. G. MacArthur and Jones ('17) have found differences in rate of respiration in different parts of the central nervous system which indicate the existence of a gradient in rate of respiration. The rate of respiration is highest in the cerebrum and decreases in the various parts in the following order: cerebellum, midbrain, medulla, corpus callosum, spinal cord, nerve. The authors find that gray matter consumes about twice as much oxygen and produces about one and one half times as much CO_2 as white matter, and some of the differences in respiratory rate in different parts of the nervous system are doubtless due to differences in proportion of white and gray matter. For example, the relatively low rate of the corpus callosum is undoubtedly associated with the fact that it consists of white matter, nerve fibers, rather than cells. But the differences in respiratory rate between cerebrum and cerebellum and between midbrain, medulla and spinal cord are scarcely to be accounted for in this way. These differences constitute highly significant evidence for the existence of an axial gradient in rate of respiration in the central nervous system.

All the evidence is in agreement as regards the existence of these physiological axial gradients. Thus far every living physiological axis examined has given evidence of the existence, at least in the earlier developmental stages of such a gradient, and in many cases the experimental methods show the presence of a gradient where structural, or other directly visible indications of its presence are absent. In whatever terms we may finally interpret these gradients, there can be no doubt concerning their existence. They are physiological facts, and their significance for localization, differentiation and functional relation is already demonstrated.

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[1] Alvarez, '14, '15*a, b*; '16*a, b*; '17*a, b*; '18*a, b, c*; Alvarez and Starkweather, '18*a, b, c*, '19; Alvarez and Taylor, '17*a, b*; Taylor and Alvarez, '17.

The Origin of the Axiate Pattern in Nature and Experiment.

It is a familiar fact that physiological axes persist through certain agamic reproductive processes and are therefore inherited by the individuals resulting from such reproduction, but in at least many eggs the axiate pattern apparently arises *de novo* during the growth of the egg. Granting that axiate pattern in its simplest form is a gradient pattern as already pointed out, we have at present no grounds for believing that such pattern is inherent in protoplasm, or that it can arise *de novo* in protoplasm apart from the action of environmental factors. Apparently a differential exposure of the protoplasm to some environmental factor or factors which affect its rate of activity, *i.e.,* which are primarily quantitative rather than specific or qualitative in their action, is necessary for the origination and establishment of an axial gradient in protoplasm. In other words, the gradients must arise through the differential action of environmental factors which affect primarily the rate of general protoplasmic activity.

Origin of Axiate Pattern in Plants and Simpler Animals.—As regards the plants, Winkler ('00*b*) and Kniep ('07) showed that in certain algæ, *e.g.,* various species of Fucaceæ, differential illumination of the two sides of the egg determines the polarity of the plant developing from the egg, though in the absence of light polarity appears and germination occurs, but more slowly. Stahl ('85) showed that in the spore of *Equisetum* polarity is determined in the same way. Winkler ('00*a*) also showed that polarity in *Bryopsis* could be determined by light. In various bilaterally symmetrical plants, such as liverworts, light determines the dorsiventrality, and some algæ develop a radially symmetrical thallus when the illumination is equal on all sides of the polar axis and are bilateral when the illumination is from one side. In the spermatophytes conditions which determine egg polarity are undoubtedly intraorganismic. The embryo sac shows a definite polarity with respect to surrounding parts, and the ovum is attached to one end of the sac. The unattached end of the ovum becomes the apical, the attached end the basal pole of the embryo. What particular factors are concerned in this case is not known.

In the simpler animals we see new polarities arise at cut ends of pieces, *e.g.*, the development of new apical regions and axes from the aboral ends of pieces in various hydroids, the development of heads from posterior cut surfaces in *Planaria.* In such cases the new axis is always represented by a new gradient, and the relation between the new axis and the occurrence of differential exposure is obvious, though whether the wound stimulus, oxygen supply, or some other factor is chiefiy concerned in determining the new gradient is not known. Loeb ('92) has maintained that new polarities are determined by gravity in the regulatory development of the hydroid *Antennularia antennina,* but Morgan ('01) and Stevens ('02, '10), while not disputing Loeb's results, showed that other factors besides gravity were concerned in determining polarity.

In the development of sponges from dissociated tissue cells described by H. V. Wilson ('07, '11) the polarity of the new individual is determined by some sort of differential between free and attached surfaces of the cell mass, the osculum developing on the free surface. Similarly in the experiments on obliteration of a preëxisting polarity and the establishment of a new polar axis in hydroids (Child, '15*c*, pp. 142–146) the apical region of the new axis arises in the region of greatest exposure to the environment, but the particular factors chiefly concerned have not been determined. In both these cases the sponge and the hydroid, the difference in oxygen supply between the exposed surface of the cell mass and the surface in contact suggests a probable factor in determining the new axis.

In the actinian *Harenactis* the localization of a region of more rapid and more extensive growth by injury is sufficient under certain conditions to determine the position and development of new polarities (Child, '09, '10*b*, '15*c*, Figs. 79–83). The axial gradation results in this case from the fact that the activity of the cells is greatest in the middle region of such an area and decreases toward its borders. In this connection a recent statement of Harrison's concerning the limb-rudiment of *Amblystoma* is of interest. Harrison says: "The limb rudiment may be thus regarded, not as a definite circumscribed area like a stone in a mosaic, but as a center of differentiation in which the intensity of the process diminishes as the distance from the center increases, until it passes away into an indifferent region. Many other systems, such as the nose, ear, hypophysis, gills, seem to have the same indefinite boundaries which may even overlap one another" (Harrison, '18, p. 456). In other words, Harrison conceives these primordia as gradients in activity in a more or less specialized cellular region of the embryo. Such gradients differ from the general axial gradients of the body only in that they are determined in some way, presumably by intraorganismic correlative conditions in specialized body regions, and are concerned with particular organ complexes instead of with the body as a whole. In still other cases new polarities are apparently determined and localized by slight differences in activity between different cells of a mass. Such differences determine the more or less definite localization of a region of growth in which the activity decreases toward the periphery and as growth progresses an axis arises. Determination of new polarities in this way apparently occurs in many cases when pieces of naked hydroid stems give rise to multiple stolons, each of which represents a new axis and a new gradient. These multiple polarities have been observed by many investigators, and I have been able to produce them experimentally in hydrozoan planulæ by first obliterating the original polarity through differential inhibition. In the origin of adventitious buds from the epidermal cells of the *Begonia* leaf similar local growth areas with gradients in activity from center toward periphery and from the surface inward are the first indications of the new plant axes (Regel, '76, Child, '15*c*, Figs. 38, 39). The localization of the new axis in such cases appears to be largely a matter of slight fortuitous differences in activity in different cells or cell groups in consequence of which certain cells or groups react more rapidly than others to the experimental conditions.

The Animal Egg.—As regards the animal egg, the evidence is very incomplete, but indicates that in at least many forms polarity is determined during the growth period of each egg by differential exposure. In various hydromedusæ for example the growing oögonia constitute a columnar epithelium, one end of each cell being separated from the exterior only by a layer of very thin flattened cells, while the opposite end is attached and adjoins the radial canal. When portions of the medusa ovaries are slightly teased to separate the eggs it is found that the free end of the egg, the end nearest the exterior, represents the high region, the attached end the low region of a gradient in susceptibility and permanganate reduction. A similar gradient appears in developmental stages and while the absence of good landmarks makes it impossible to demonstrate that the later gradient is identical with the earlier, there can be little doubt that it is. In the sea urchin the oögonium is attached to the wall of the ovary at one small region of its circumference and here also, as Boveri has shown, the free pole becomes the apical pole and represents the high end of a gradient. In these cases it is not the pole through which nutrition enters, but the unattached pole which in the medusa is more exposed to external factors and in the sea urchin to the fluids of the ovary which becomes the apical pole. It seems probable that a differential in oxygen supply and perhaps also in CO_2 concentration are chiefly concerned in determining the polarity and the gradient which represents it in these cases. Even in the worm *Sternaspis,* in which a peduncle containing a vascular loop develops in connection with each growing oögonium, the attached pole of the egg, into which the vascular loop enters becomes the basal pole.

In the eggs of the higher animals where oxygen as well as nutritive substances reach the egg chiefly or wholly through the blood, the polarity may apparently be determined by relation to the blood supply. Bellamy ('19) has shown that in the frog's egg polarity apparently develops in definite relation to the vascular supply, the apical pigmented pole arising on the arterial, the basal unpigmented pole on the venous side. In these cases the oxygen supply or the conditions determining rate of respiratory exchange are apparently the chief factor in determining polarity, the region of most rapid exchange becoming the apical pole.

Symmetry in animals, like polarity is indicated by gradations in physiological condition, and although a particular kind of protoplasm may give rise normally to a radial or a bilateral animal, experiment shows, that for certain forms at least, the normal symmetry of pattern is not inherent and unchangeable. In the radial anemone *Harenactis,* for example, bilateral tentacle groupings may arise under certain experimental conditions (Child, '09) and it is possible through differential inhibition to obliterate bilaterality and produce radial larval forms in the sea urchin (Child, '16*d*) and also in the starfish (unpublished). Moreover, in pieces of *Planaria,* under experimental conditions which practically obliterate the polar gradient the symmetry gradient may become the polar axis of the new individual (Child, 15*c*, pp. 163–165).

A characteristic feature of radial symmetry is the repetition of parts, usually axiate and often bilateral, in pattern about a center. Such repetition must be largely a matter of the space relations of specialized growth centers. Each growth center involves or dominates a certain area, and only at a certain distance from it can another similar center arise. Thus the number of such growth centers arising on a given circumference depends on the area dominated by each center which varies with physiological condition and on the size of the circumference and can be altered experimentally in many cases. Moreover, as growth in size of the circumference occurs and the distance between the repetitive parts increases we often find additional new parts arising, *e.g.,* mesenteries and tentacles in many actinians, etc. Such processes are physiologically similar to many forms of agamic reproduction, being essentially reproductions of specialized parts instead of new wholes, resulting from physiological isolation (Child, '15*c,* Chaps. IV., V.).

The Problem of Symmetry.—Bilaterality is inherited through many processes of agamic reproduction, *e.g.*, in flatworms and annelids and may of course also be inherited in many eggs. On the other hand, it may conceivably be determined in some eggs by ovarian conditions, by conditions connected with maturation or with fertilization, or perhaps even by conditions arising later in development. Moreover, many bilateral forms develop characteristic asymmetries during the course of development, *e.g.*, the asymmetry of gasteropod mollusks and the visceral asymmetry of vertebrates, or a well-developed bilateral symmetry may give rise in metamorphosis to a radial-bilateral pattern of very different kind, as in certain echinoderm groups. Our knowledge concerning the physiological aspects of the origin of symmetry in animals is still very fragmentary, but the earliest indications of the presence of a particular symmetry pattern are gradients in physiological condition, which, so far as the evidence goes, are similar to the polar gradients, and in plants we see the different symmetry patterns arising through differential exposure to the action of external factors. In the light of all the facts we are justified in concluding that even though a particular symmetry pattern may persist through reproduction, *i.e.*, be inherited in a particular case, symmetry like polarity must in the final analysis arise through differential exposure to the action of external factors. On the other hand, even if we grant that the differential exposure of the egg of the medusa, the sea urchin, *Sternaspis*, etc. (see p. 173), determines the polarity, and that symmetry may also be determined by relation to environment, it is evident that, except in some of the simpler organisms, the differential exposure of the egg is not fortuitous, but is determined by the hereditary mechanism of the organism. The epithelial arrangement of eggs in the medusa gonad, the position of the sea urchin egg in the ovary, the development of the peduncle and the vascular loop in *Sternaspis*, and the circulatory pattern in the chorion of the frog's egg are all features of the hereditary mechanism of the organism concerned. Even in such cases then, as well as in cases where the axiate pattern persists through reproduction, the hereditary mechanism is concerned in the origin of the axiate pattern of the new individual. Moreover, even in the case of *Fucus* where the determination of polarity by the direction of incident light is apparently wholly fortuitous, the hereditary mechanism, as expressed in the constitution and pattern of the egg protoplasm, determines the occurrence and the nature of the reaction to incident light. In other words the hereditary constitution of the *Fucus* egg as well as the direction of incident light is a factor in the determination of the polarity. There is, in short, no conflict between this physiological conception of the origin of organismic or of axiate pattern and conceptions of heredity. The origin and development of organismic pattern in nature is simply the realization of certain hereditary potentialities of a particular protoplasm in a particular environmental complex, which may itself be determined in large measure or wholly by the hereditary mechanism of the protoplasm concerned.

Surface-interior Pattern and Axiate Pattern.—In the light of the conclusions reached concerning the nature and origin of axiate pattern, the question of cell pattern, touched upon above (pp. 150–151) requires some further consideration. It was suggested that the cell is primarily a surface-interior pattern resulting from exposure of the surface of a mass of protoplasm to the action of external factors. Such an exposure is a differential exposure as regards surface and interior. Both the respiratory exchange and excitation can occur only through the surface, therefore differences must arise between surface and interior, and a more or less definite gradient in such conditions from the surface inward must result. As different organs are localized at different levels of an axial gradient so the localization and differentiation of the nucleus in the first instance may have resulted from the conditions in the interior of the protoplasmic mass. In fact, it is difficult to see how the nucleus as a definite organ could have arisen otherwise. The differences between nucleus and cytoplasm as regards acidity and electric potential as well as the behavior of nuclei in such specialized cells as spermatozoa, where cytoplasm is practically absent, all suggest that the nucleus is fundamentally an internal cell organ, and if the origin of cell pattern has any relation to environmental factors, the differentiation of the nucleus must have been determined originally by conditions in the interior of a protoplasmic mass. The fact that the nucleus persists from one cell generation to another means merely that the pattern once established is persistent or inherited, although it is difficult to determine to what extent the persistence of the surface-interior conditions is concerned in the persistence of the pattern.

Viewed from this standpoint, cell pattern originates in the differential between surface and interior in general, and axiate pattern in differential between different parts of the surface of the protoplasmic or cell mass concerned. As regards the axiate pattern, the evidence indicates that the differential is primarily quantitative and involves differences in the rate or degree of fundamental protoplasmic activity, but as regards cell pattern we have at present no means of determining whether the differential was primarily quantitative, though various lines of evidence point in that direction.

The presence of an axiate pattern does not imply the disappearance of a general surface-interior pattern, either in the cell or the multicellular organism. All organisms show some kind of surface-interior pattern at least in the superficial regions of the body, and all the facts indicate that in the final analysis such pattern arises through exposure of the surface. The passage of cells to the interior of the embryo by gastrulation is of course a feature of axiate pattern, but conditions in the interior are undoubtedly factors in determining the further differentiation of such cells into the organs of entoderm and mesoderm. Only in some of the simpler animals does the general surface-interior differentiation arise *in situ*. Formation of entoderm by delamination in all cells of a blastula for example appears to be a case in point, and in the protozoa definite structural differentiation is limited to the ectoplasm. But in the ectoderm of multicellular animals, for example, we find numerous evidences of surface-interior pattern ranging from the basal muscular extensions of ectoderm cells in *Hydra* to various complicated sensory structures and the early differentiation of the neural tube in the higher animals, and the relation of such features of pattern to exposure of the surface appears obvious. Here, however, as in the case of the axiate pattern, the surface-interior relation represents merely the physiological conditions under which the potentialities of the hereditary mechanism of the protoplasm are realized. The fact that the embryo possesses a surface determines certain relations in the protoplasm to this surface and the specific constitution of the protoplasm determines the kind of reaction which occurs, the sort of specialization which develops. If an axiate pattern is also present, physiological conditions are provided for differences in reaction in relation to the surface at different regions or levels of the body. This of course does not mean that exposure to a special external stimulus is necessary for the development of a particular superficial organ. Light, for example, is not necessary for the differentiation of a photoreceptor. The surface-interior relation merely determines that the physiological conditions under which the hereditary potentialities of a given protoplasm to produce a photoreceptor are realized are conditions which arise in development at or near the surface of the developing organism.

The Gradients in Relation to Excitation and Transmission.—The differential exposure of the cell or cell mass to the action of environmental factors is only the first step in the establishment of the gradient. Admitting that this differential exposure determines a higher rate of activity in some region, we may expect, since living protoplasm is irritable, and since increased activity in one region serves to some extent to excite adjoining regions, that transmission from the region of increased activity will occur. In the absence of highly specialized conducting paths we find that protoplasmic excitation apparently undergoes a decrement in intensity or effectiveness with increasing distance from the point of origin, so that an excitation gradient results. Discussion of the evidence bearing on this point is impossible in this paper, but many facts indicate that the physiological relation resulting from the differential exposure of a cell or cell mass is primarily a relation of excitation and transmission and that the resulting gradient is essentially the fixation or establishment of an excitation-transmission gradient in the protoplasm through the modification of the protoplasmic substratum by the persistence or repetition of the differential exposure. From this viewpoint the gradient represents the most primitive sort of excitation-transmission relation and its effects upon protoplasm.

In a series of recent papers, R. S. Lillie has advanced a theory of excitation and transmission[1] in terms of the plasma membrane, its electrical depolarization in excitation with increase in permeability and resulting electric current. The membrane changes in excitation are conceived by Lillie as involving a chemical reaction, probably oxidative, and the electric current resulting from the depolarization at any point is regarded as the agent of transmission, since it tends to produce depolarization at other points within a certain range and so induces excitation at those points.

Lillie's theory seems to me to account very satisfactorily for a wide range of observed facts, particularly in the more highly specialized excitations and transmissions, but the question may be raised whether the depolarization of the membrane is always the primary factor in excitation or the chief source of the electric current. A region of rapid oxidation gives rise to an electric current similar to that which Lillie regards as arising from the depolarization of the membrane. Doubtless in protoplasm membrane changes and oxidations are not independent, but while the highly specialized process in the nerve may be very largely or wholly a membrane process, it seems probable that in the primitive excitation processes the oxidations play a more important, perhaps the chief part, and that the excitation is not necessarily limited to the plasma membrane. In his later papers Lillie apparently recognizes and admits this probability.

As Lillie points out, the strength of the current produced at a point of excitation decreases with increasing distance from that point, because of resistance, and it can act as a stimulus only within a certain distance. In other words, this agent of transmission undergoes a decrement or represents a gradient from the point of original excitation, and except in highly specialized tissues which react according to the "all or none" law, the current must determine decreasing degrees or amounts of change and so of excitation at increasing distances from the point of original excitation. Even though these new excitations give rise to sufficient current to excite points beyond the range of the original current the transmission process will show a decrement and finally cease. Briefly, this means that the primitive excitation-transmission relation in protoplasm must be a gradient with decrement from the region of most intense activity.

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[1] R. S. Lillie, '09*a*, *b*, *c*, '11, '13, '14, '15, '16*a*, *b*, '17, '18, '19; R. S. Lillie and E. N. Johnston, '19.

We are accustomed to say that excitation is completely reversible, and as regards the nerve fiber it may be true, but certainly memory and the possibility of learning demonstrate that at least in certain parts of the nervous system reversibility is not complete. Similarly in muscle the contraction is reversible, but repeated excitation within limits brings about growth, *i.e.,* functional hypertrophy. The fully developed organism has reached or approaches a condition of dynamic equilibrium in which most changes except the progressive changes of age are relatively reversible. Most of the less readily reversible changes have already occurred during development. But embryonic protoplasm stands at the beginning of the developmental process of equilibration, and while the momentary and more superficial excitations are undoubtedly largely or wholly reversible, conditions which persist for any considerable length of time must produce modification in the protoplasmic substratum and this may be more or less permanent, according to the nature of the protoplasm. According to this conception, then, the physiological gradients arise as primitive excitation-transmission relations in protoplasm, and if the differential exposure persists long enough they become more or less persistent and constitute physiological axes or radii.

Objection to this conclusion may be raised on the ground that oxygen supply or rate of removal of CO_2 which have been regarded as factors concerned in determining gradients are not properly speaking "stimuli" but rather specific material relations between portoplasm and environment. As a matter of fact, however, we know that sufficient differences in oxygen supply or in rate of removal of CO_2 do determine differences in the rate of the respiratory activities of protoplasm, and the respiratory activities are apparently essential factors in excitation, particularly in the more primitive excitation processes. As already suggested, many facts indicate that excitation in highly specialized protoplasms with special structural mechanisms may differ more or less widely from the process as it occurs in protoplasm in general, but we are concerned here rather with the process in its most general terms than with such specialized processes. Excitation in the broad sense is apparently an increase in the rate of living or at least of that aspect of life which concerns energy liberation, and the respiratory relations to environment are factors in determining that rate. If a region of higher rate of respiratory activity is determined in a cell or cell mass by differential exposure to oxygen or to removal of CO_2 that region is in a state of excitation as compared with other regions and the relations between it and such other, regions are primarily those which exist in general between excited and unexcited regions. Oxygen supply and CO_2 are then not only specific material factors in the relation between protoplasm and environment, but they are or may be quantitative factors in this relation.

From the first moment when differences at different levels of the gradient begin to arise, the possibility of chemical or transportative correlation arises, and as the progressive complication occurs and different substances appear in the protoplasm at different levels, these possibilities become always increasingly varied complex and specific. On the other hand, it is evident that definite and orderly chemical or transportative correlation as between protoplasmic regions rather than protoplasmic constituents cannot occur until definite and orderly regional differences are present. The gradient is merely the first step in the development and evolution of axiate pattern, and the progressive complications and alterations of the relations and changes initiated by its appearance are terminated in the individual only by agamic reproduction or some other process involving dedifferentiation and reorganization, or by death, and as regards its evolutionary termination, even speculation is idle.

Conclusion.

It is perhaps necessary to point out that the conception with which this paper is concerned, the conception of axiate pattern as primarily a quantitative gradation in physiological condition, is nothing more than an attempt to interpret certain aspects of the physiology of development. The axial gradients do not create anything, they are not the "cause" of growth or differentiation in the organism, they do not determine what organs shall develop in a particular protoplasm. Granting their existence and the significance which I have assigned to them they represent merely certain physiological conditions, under which the hereditary mechanism of a protoplasm gives rise to the order or pattern which we call axiate or axiate-symmetrical.

The egg in most cases requires fertilization or the action of some other factor external to it to initiate development, but the specific hereditary constitution of the egg protoplasm with its potentialities of development is present, whether fertilization or initiation of development by other means occurs or not. Physiologically speaking the spermatozoön or some other factor external to the egg merely sets the mechanism in motion or gives it the necessary speed and development proceeds. Similarly the gradient in the egg, whether it persists from earlier cell generations or arises anew in the egg through differential exposure, is merely a physiological condition which determines that the hereditary mechanism shall give rise to a particular order or pattern. Alteration of the gradient relations alters the pattern though the hereditary mechanism remains the same. The gradient is then nothing more than one of the physiological conditions under which the development of axiate organisms occurs, and the surface-interior relation, whether a gradient or not, is merely another even more general physiological condition of organismic development.

The idea of a quantitative gradient in physiological condition as the condition initiating axiate development and of its origin in the final analaysis through the action of an external factor does not require or depend upon any particular assumptions or theories concerning inheritance or evolution. Some of the critics of the conception have regarded it as Lamarckian, but it is not, though of course it might be used if desired in a Lamarckian way. Strictly speaking, it has nothing directly to do with either inheritance or evolution, except in so far as it maintains that axiation or polarity and symmetry are not inherent properties or characteristics of protoplasm. It is fundamentally a physiological conception formulated on the basis of many different lines of observational and experimental evidence, and while its formulation in the present state of our knowledge is necessarily incomplete and perhaps vague, I believe a fair consideration of the evidence now available justifies the conclusions advanced.

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EXPERIMENTAL STUDIES ON MITOCHONDRIA IN PLANT CELLS.

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Introduction.

During the last few years several investigations have been carried on with a view to utilizing the mitochondria as cytoplasmic indicators of cell activity. Reasoning on the basis of the fundamental differences which exist between the mitochondria and the nucleus it has been natural to assume that they may serve as clues to different types of activity. We are accordingly faced with the possibility of studying physiological and pathological processes from an entirely new angle, which may yield valuable and unexpected information. Thus far the results obtained from this new line of work have been contradictory and difficult to explain. On the one hand we have indications that, in certain instances, the mitochondria are very labile and respond easily to alterations in cellular activity (Busacca, '15; the Lewises, '15; Homans, '15; Scott, '16; and Goetsch ,'16); and, on the other, equally convincing observations to the effect that, in other cases, they are fixed and permanent cytoplasmic structures which do not evidence distinctive variations even under the influence of fairly advanced pathological conditions (Clark, '14; Strongman, '17; McCann, '18; and Rasmussen, '19, etc.). This condition has filled some investigators with enthusiasm and discouraged others. It should, however, only act as an incentive toward a systematic experimental study of mitochondrial variations.

Since the substantial similarity of mitochondria in plant and animal cells has already been demonstrated (N. H. Cowdry, '17) we have ample material to choose from. It is possible to apply certain experimental conditions to plants which are not feasible in the case of animals, and *vice versa*. For the purposes of this paper I have selected the sprouting rootlets of peas because the normal properties of the mitochondria are well known and on account of the fact that the cells are relatively simple, owing to the absence of photosynthesis and extensive starch formation. In view of my failure to find any observations in the literature dealing with the experimental study of mitochondria in plant cells, I have planned my experiments so as to embrace a wide range of conditions in order to be able to define specific problems for further intensive study.

Material and Methods.

After the seeds had been sprouted and the plantlets experimented with, the radicles were treated by the Regaud formalin-bichromate method, and the sections (cut 4μ) were stained with iron hematoxylin. It is of the utmost importance to use good and fresh formalin. In order to neutralize the free formic acid, which is so often present, I am in the habit of adding a small amount of magnesium carbonate to the stock solution. Attempts were made to control this method through the observation of living cells supra-vitally stained with janus green without very satisfactory results owing to the difficulty of getting the dye to penetrate the cells.

Since mitochondria exhibit considerable variations in different parts of the radicle I have confined my observations to the cortical cells between the elongating part and the root-cap. Even in this portion, however, neighboring cells occasionally exhibit, without apparent reason, variations in the size, shape and general appearance of the mitochondria, calculated to lead the unwary astray, and the cells near the surface react to experimental conditions and to fixation differently from those more deeply placed. It is important, also to bear in mind that mitochondria show a marked and constant regional variation in different parts of single cells so that serial sections are necessary for their study.

The usual appearance of the mitochondria in the cells under investigation is illustrated in Fig. 1. They will be seen to occur in the form of granules, short rods and filaments which show no indication of plast formation. They are distinctly more filamentous in the cytoplasm immediately beneath the cell membrane than in the region of the nucleus; this, however, is not so well shown in the figure. The mitochondrial filaments near the nucleus show a tendency to become swollen and to stain more intensely with iron hematoxylin throughout their whole length or in certain restricted areas, as contrasted with those in the more peripheral parts of the cell.

The vacuoles occupy a considerable portion of the cytoplasm and sometimes contain a few mitochondria in their interior. These mitochondria, which are always darkly stained, enlarge into spherules of different sizes.

The effect of different experimental conditions was studied as follows:

Observations.

1. *Centrifuging.*

Plantlets were centrifuged for one hour with results as indicated in Fig. 2. The nucleus appears to have been thrown against the cell wall and is in some cases quite flattened. While a thin layer of protoplasm remains everywhere in contact with the cell wall, the greater portion of it has assumed a postion between the nucleus and the vacuole. It is of shreddy consistency and contains enlarged mitochondria.

I have seen no indications of the existence of a difference in specific gravity between the mitochondria and the protoplasmic ground substance in the cortical cells of the pea radicle as described by Fauré-Fremiet ('13, p. 602) in *Ascaris*. Key,[1] using also the centrifuge method, failed to detect any difference in the specific gravity of the mitochondria and the protoplasmic ground substance of nerve cells. On the other hand Beckwith ('14, p. 216) succeeded in separating the cytoplasmic constituents in the eggs of *Hydractinia* into three layers: first, the oil cap, second, a layer of clear protoplasm and, lastly, a mingled mass of yolk and mitochondria. These observations indicate, probably, the existence of variations in the fluidity of the ground substance, not of differences in the mitochondria themselves.

2. *Plasmolyzing Agents.*

Normal plantlets of two days growth were placed so that their radicles were immersed in a 20 per cent. solution of cane sugar for 48 hours. The resulting plasmolysis is illustrated in Fig. 3. It will be noted that the mitochondrial filaments have almost completely disappeared leaving only a comparatively few enlarged spheres, sometimes with faintly staining centers. The vacuoles show no mitochondrial content. None of the control peas subjected to the same treatment survived and the assumption is, in agreement with Guilliermond ('18), that the mitochondria enlarged after the death of the cell.

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[1] Quoted from E. V. Cowdry ('18, p. 84).

3. *Desiccation.*

Normal plantlets were deprived of water at room temperature for 36 hours, which resulted in considerable shrinkage of the growing root-tips. On examination the cells were found to contain for the most part granular, and rather swollen mitochondria (Fig. 4). The vacuolar membrane seems to have disappeared and the protoplasm exhibits great variation in intensity of staining reaction, as do also the contained mitochondria, which in other respects appear to be quite normal.

4. *Illumination.*

Some plantlets were kept in complete darkness, while others of the same age were subjected daily to strong sunlight without affecting in any distinctive way the mitochondrial content of their radicles. It would seem, therefore, that the mitochondria in the cortical cells of the pea radicle are not directly concerned with chemical processes which are accelerated and retarded by variations in illumination. Unfortunately the chlorophyll-containing plumules were not examined.

5. *Increased Temperature.*

Plantlets were heated in a thermostat at various temperatures, care being taken to avoid evaporation.

The first changes were observed in radicles exposed to a temperature of from 43 to 46 degrees C. for 3 hours (Fig. 5). All the filamentous mitochondria disappear and their place is taken by numbers of small faintly staining granules, though a few large and deeply staining spherules may still be seen. It is possible that this segmentation of the mitochondria might not have occurred had the temperature been applied gradually instead of suddenly.

When heated to a temperature of from 47 to 49 degrees C. for 30 minutes the above mentioned granules show a darker stain and appear fewer in number (Fig. 6). Many peculiar, irregular, darkly staining masses may be seen within the nuclei which are difficult to interpret. Controls showed that the tips were all killed, but the remaining portions of the plantlets recovered in a few cases.

When exposed to a temperature of from 65–73 degrees C. for 40 minutes the intensely staining granules become greatly reduced in number (Fig. 7), the cytoplasm varies greatly in consistency and staining reaction, and the nuclei lose all traces of deeply staining nucleoli and granules. None of the controls of the experiment survived.

In my experience, therefore, plant mitochondria are not rendered vesicular through increase in temperature in the same way as the mitochondria in the tissue cultures experimented with by the Lewises ('15). I have also been quite unable to confirm Policard's ('12, p. 229) interesting observation that in some glandular cells the mitochondria become partly dissolved, when heated to a certain temperature, leaving an unstained residue behind which he believes to represent their albuminous component. According to Jost ('07, p. 140), however, albumins proper occur only occasionally in plant cells.

6. *Decreased Temperature.*

Plantlets were exposed to a temperature of 11 degrees C. in an ordinary ice box for 4 days and preparations were made of their radicles. The cells showed very little change (Fig. 8) ; but it was possible to detect a slight tendency toward clustering of lightly stained filamentous mitochondria in the perinuclear area, and further, that these filaments do not enlarge and stain more intensely than those in other parts of the cytoplasm as they do in normal cells. The experiments which I have already mentioned on the effect of illumination preclude the possibility of attributing this change in the mitochondria to the darkness of the ice box.

An exposure to the same temperature for eighteen days apparently brings about a breaking up of the mitochondrial filaments into short rods and granules which stain intensely (Fig. 9). The protoplasm is also seen to be distinctly reticulate, the mitochondria occurring in the denser strands. The more intense staining of the nucleolus, shown in the figure, appears to be an individual variation not associated with exposure to cold.

Other plantlets which were placed in a freezing mixture of ice and salt in the ice box for 20 hours show more advanced alterations (Fig. 10), the mitochondria remaining about the same and the protoplasm showing many scattered vacuoles, the reticulate appearance having disappeared.

The presence of distinctly filamentous mitochondria after a sojourn of 4 days in the ice box tends to support Dubreuil's view ('13, p. 137) that filamentous mitochondria are indicative of rest and granular ones of active multiplication by division, because it is safe to assume that the chemical changes in which the mitochondria are concerned share in the general retardation occasioned by reduced temperature. Other important considerations however show that this generalization does not hold (E. V. Cowdry, '18, p. 67).

7. *Submergence in Water.*

Entire plantlets were submerged in ordinary tap water for 24 hours and thus deprived of the regular amount of oxygen. The chief alterations are manifest in the inner cells of the cortex (Fig. 11), in which the mitochondria have broken up into rather large short rods and granules which vary in their staining reaction. The more superficial cells of the cortex, on the other hand, show no characteristic changes, the mitochondria retaining their normal filamentous shape. The controls showed a varying degree of vitality, about 50 per cent. being killed.

8. *Restricted Air Space.*

Plantlets were placed in a bottle, containing an air space of about 15 c.c. and tightly closed with a paraffin-coated cork. Preparations were made after 24 hours which showed a reversal of the changes due to submergence; for in this case the alterations were more marked in the outer cortical cells, which present complete chondriolysis, than in the deeper ones. In the middle cells of the cortex the mitochondria exhibit great polymorphism as illustrated in Fig. 12, rings, spheres, rods, granules, and dumb-bell shaped forms being visible. Variations in staining reaction are of common occurrence and the mitochondria have lost their tendency to cluster about the nucleus. The inner cells, on the contrary, contain the usual thin filaments with some plast-like forms (Fig. 13).

When the same treatment is continued for two days the alterations become still more pronounced. The cells of the plerome are illustrated in Fig. 14. The mitochondria are very scanty, the protoplasm shows signs of disorganization and the nuclei are loaded down with a granular deposit. The controls from this series all died so that we are unquestionably dealing with death changes.

These observations seem to be in accord with the view first advanced by Kingsbury ('12, p. 46) and supported by Mayer, Rathery and Schaeffer ('14, p. 619) that the mitochondria play an active part in protoplasmic respiration.

9. *Chloroform.*

Plantlets were exposed to the vapor of chloroform, in a covered Petri-dish of 100 c.c. capacity for 45 seconds without the mitochondria undergoing any marked alterations. Continuing the same treatment for 2½ minutes, however, brings about distinct changes (Fig. 15), only a thin layer of protoplasm remaining intact, in which may be distinguished a few scattered mitochondria with very vague outlines. The vacuole is filled with an intensely stained granular deposit without any trace of mitochondria. Plasmolysis is fairly advanced. This treatment resulted in the death of the root tips of the control plantlets. It is interesting to compare this condition to the well known immunity of mitochondria to the action of chloroform after fixation.

10. *Ether.*

On exposure to the vapor of ether, under the same conditions, for 3½ minutes no distinctive changes were observed in the mitochondria. After exposure for seven minutes the mitochondria in the cortical cells are seen to be shrunken and of irregular outline, and to be darkly stained (Fig. 16). Peculiar, irregular, darkly staining masses also appear in the nuclei which are difficult to interpret. The protoplasm has changed in consistency in the cells, but in those of the early meristem and root-cap it is shrunken and stained uniformly and intensely in these preparations, so that the internal structure is difficult to make out. This is not due to incomplete differentiation. The cell walls in these regions seem to have undergone partial fragmentation.

Controls subjected to the same ether treatment and afterwards placed on damp absorbent cotton grew vigorously with the plumule much greater than in the case of other peas sprouted at the same time which had not been treated with ether. According to Jost ('07, p. 195) weak etherization accelerates respiration while strong etherization inhibits it, by killing the cells. It will be seen that growing the plantlets in lecithin has the reverse action in stopping the growth of the plumule abruptly and in inhibiting chlorophyll production.

The mitochondria are if anything less affected by 7 minutes in ether vapor than by 45 seconds exposure to vapor of chloroform.

11. *Glycerin.*

Plantlets were, as usual, sprouted normally and then placed in a 10 per cent. solution of glycerin in tap water for 18 hours. This treatment brings about a complete chondriolysis of all the mitochondria in the root tip (Fig. 17) and may be compared with the effect of extreme heat (Fig. 7) and of restricted air space for two days (Fig. 14), though the condition of the ground substance is somewhat different in these conditions. None of the controls survived.

12. *Lecithin.*

Plantlets were placed so that their radicles were in intimate contact with sphagnum moss, thoroughly soaked with a 1 per cent aqueous solution of lecithin, for 24 hours. (The lecithin used was a pure variety obtained from Dr. Levene of the Rockefeller Institute.) The cells in these preparations present a remarkable picture (Fig. 18). They are loaded with a multitude of granules and filaments, some of which appear to be swollen and elongated to a relatively large extent, indicating perhaps imbibition of lecithin from the surrounding fluid and subsequent incorporation in the mitochondrial substance. It is to be further noted that these enlarged mitochondria are never truly vesicular; their staining reaction, in some cases, appears to be fainter than that of the definitive mitochondria, which presumably have not approached the nucleus and which are changed to a comparatively minor degree.

Other plantlets grown on blotting paper saturated with lecithin solution exhibited the same appearance.

Radicles, of control plantlets subjected to the same treatment, grew vigorously, but the growth of the plumule was abruptly stopped and chlorophyll formation ceased entirely. Compare this with the growth and increased chlorophyll production under the influence of ether.

This association of lecithin with the enlargement of mitochondria is of interest from several points of view. In the first place, Russo ('12, p. 215) claims to have been able to increase the mitochondria in the oöcytes of the fowl through the injection of lecithin. Unfortunately, however, the technique which he employed is open to criticism on account of the lack of specificity of his staining reactions. In any case, the parallelism between his observations and my own is interesting and may be significant. Moreover, Löwschin ('13, p. 203; '14, p. 269) is credited with the making of artificial mitochondria with lecithin in different salt and albumin solutions, which in some respects resemble true mitochondria very closely. Many other observations might be cited which indicate that mitochondria are allied in composition to substances of the phosphatid group.

Discussion and Conclusion.

In considering the effect of various experiments on mitochondria we must bear in mind the normal variation. They vary in diameter, length and appearance in the same cell and in different parts of the same root-tip where the cells of the plerome, periblem, root-cap, epidermis and meristem each have some peculiar mitochondrial appearance. The elongated cells of the periblem are marked by mitochondria differing from those of the younger cells of the same portion. It is impossible to find two rootlets, although growing under precisely similar conditions, in which there is not some difference in the mitochondrial contents of cells from similar portions.

We find also that there is much variation in the manner in which mitochondria react to experimental conditions in different parts of the same rootlet and also in rootlets of different stages of growth. The thin, lightly stained filaments, rods and granules are changed by many causes, the granules of older cells are more resistant, and spheres sometimes remain in the protoplasm when all traces of mitochondria have disappeared.

Segmentation is peculiar to mature filaments and occurs from many causes. It is even an individual variation in plantlets grown under precisely similar conditions. I have seen no indication that the resulting granules elongate to form new filaments or that mitochondria increase through elongation of original granules and the segmentation of the resulting filament.

Mitochondria normally agglutinate and form lipoidal masses in close proximity to the nucleus. These masses seem to disappear and to go into solution in older cells which contain only the persistent granular mitochondria. Scott ('16, p. 249) has also observed agglutination of mitochondria in the cells of the pancreas of animals poisoned by phosphorus.

Mention should here be made of the spherical inclusions so commonly seen in the vacuoles of early cortical cells, the origin of which is, in all probability, mitochondrial. They resemble very closely the spheres in cells at the base of the rootlet which clearly arise from the dissolution of aleurone grains. They both disappear in a similar manner in the median portion of the rootlet, at a distance from their origin. They probably are very similar in composition, for aleurone grains contain all the elements supposed to exist in mitochondria and probably are the source of the continuous supply of the mitochondrial matter supplied by the cotyledons.

The composition and consistency of the protoplasm has some effect on the behavior and appearance of mitochondria in normal as well as under experimental conditions. MacDougal (p. 199) in referring to the living matter of plant cells, says: "Living matter is composed mainly of pentosans and albumins, or albumin derivatives with lipins as a minor component. The proportion of the main components may vary from nearly one hundred per cent, to nearly zero." In reticulate or alveolate protoplasm, mitochondrial granules are found in the strands of the network or in the more condensed portion of the protoplasm and never in the meshes or alveoli. Filaments, on the other hand, are seen only where the "main components" are intimately mixed and the protoplasm appears to be homogeneous. The differences in chemical constitution, structure of the cell wall and in other particulars, make it difficult to compare the reactions of plant and animal cells to experimentation.

In reviewing the several experiments it will be seen that mitochondria are changed to an abnormal degree only under severe conditions which either kill the cell or render its recovery very improbable. Even when the cell has been killed its general appearance varies in almost every case, the mitochondria however are very much reduced in number or disappear entirely by apparent solution, or in the case of plasmolysis through the prior appearance of vesicles.

In the experiments with submergence and restricted air space, where a sufficient supply of oxygen for respiration has not been supplied, the mitochondria are changed in a somewhat similar manner but the location of the changes is reversed. Exposure to the vapors of ether and chloroform produce very different results, and even in cells stimulated by ether vapor or by lecithin, mitochondria are very different in appearance, though increased growth results in both cases.

I hope in my next paper to make an intensive study of the influence of lecithin upon the mitochondria in plant cells.

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EXPLANATION OF PLATES.

All the figures have been drawn with the aid of a Zeiss apochromatic 1.5 mm. objective, a Spencer ocular No. 10 and a camera lucida.

Cortical cells, between the root-cap and the elongating portion of the sprouting pea radicle, were selected. The radicles were fixed by the Regaud formalin-bichromate method and the sections stained with iron hematoxylin.

PLATE I.

FIG. 1. Normal cells of the cortex near the meristem of a radicle about 10 mm. long. The mitochondria are filamentous and granular, and exhibit a varying intensity of staining reaction. They show little tendency to cluster around the nucleus or to enlarge. The vacuoles contain mitochondrial granules.

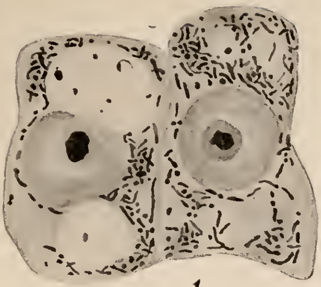
FIG. 2. Showing the effect of centrifuging for one hour. The radicle is about 6 mm. long and has not yet elongated. Mitochondria are few and unaffected in position in the protoplasm and in the vacuoles they are evenly distributed among protoplasmic shreds. The heavy nucleus adjoins the cell wall while the greater part of the protoplasm lies between the nucleus and the vacuole. The continuity of the protoplasmic lining of the cell wall is unbroken.

FIG. 3. Plasmolysis caused by immersion of the radicle, about 7 mm. long, in a 20 per cent. solution of cane sugar, for two days. Mitochondria are few in number and have enlarged into spherules with centers lightly stained. The protoplasm is very unevenly stained and contains no vacuoles. Intensely stained masses appear at the periphery of the nucleus.

FIG. 4. Illustrating the effect of drying the plantlet for 36 hours. Cell from a radicle about 9 mm. long. The mitochondria, differing in size and intensity of coloration, are granular and show no inclination to approach the nucleus. The nucleolus is only faintly stained. Other cells show a large vacuole with globules of protoplasm and many mitochondrial spheres.

FIG. 5. The plantlet was heated to a temperature of from 43 to 46 degrees C. for three hours. The radicle is short, being only 5 mm. long. Mitochondrial granules are very numerous and lightly stained. The plast-like forms are characteristic of the Alaska, a green variety of pea. The protoplasm is uniform but, where thin, it becomes alveolate.

FIG. 6. Plantlet exposed to a temperature of from 47 to 49 degrees C. for 30 minutes. Cells from a radicle 9 mm. long. Mitochondria are few and granular with increased intensity to stain. The strongly stained masses at the periphery of the nucleus probably result from the increased temperature.



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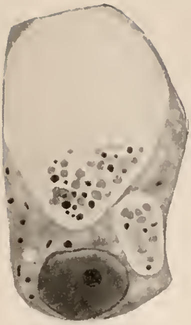

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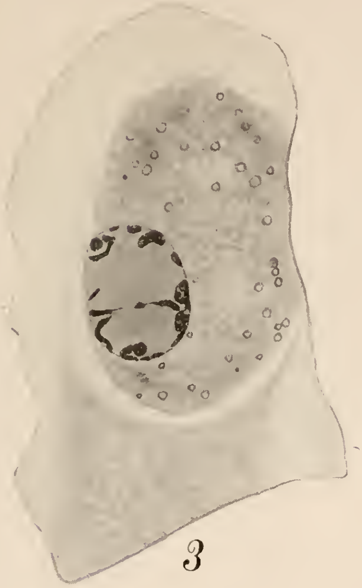

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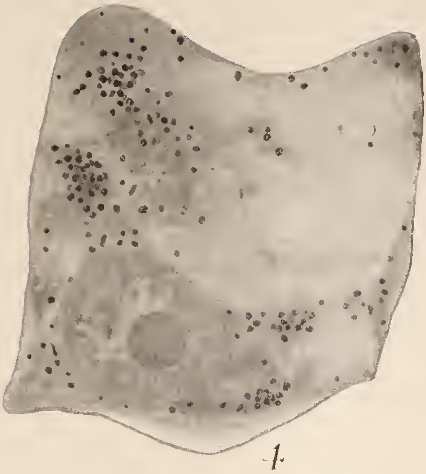

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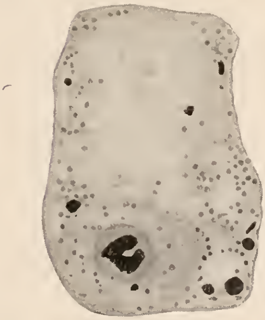

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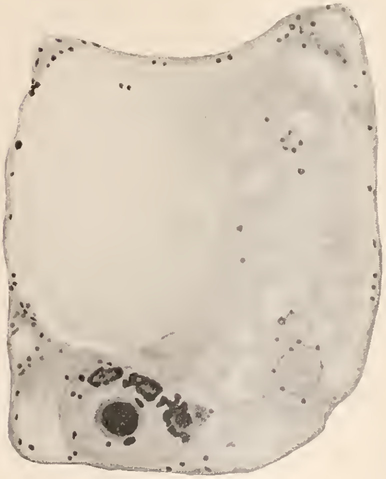

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Plate II.

Fig. 7. A plantlet of the marrowfat variety was heated to a temperature of from 65 to 73 degrees C. for 40 minutes. A cell from a radicle 10 mm. long is figured. A few mitochondrial granules still persist with others not so strongly stained and almost indistinguishable in the granular or mealy protoplasm. The black granules at the periphery of the nucleus have lost their stain.

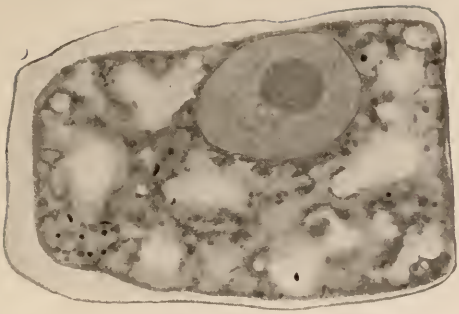
Fig. 8. The plantlet was exposed to a temperature of about 10 degrees C., in an ice box, for 4 days. The cell figured is from a radicle 9 mm. long and shows filaments, not strongly stained, clustering about the nucleus and remaining unchanged, although some show strongly stained nodes. The vacuoles in other cells contain intensely stained and enlarging mitochondrial spheres and shreds of protoplasm.

Fig. 9. Showing effect of prolonged exposure to a temperature of about 10 degrees C., in an ice box, for 18 days. The cell figured is from a radicle 6 mm. long. The filaments have, in most cases, segmented into intensely stained granules and short rods. Those in the vicinity of the nucleus are enlarged. The protoplasm is distinctly reticulate.

Fig. 10. Exposed to the low temperature of a freezing mixture of ice and salt and gradually restored to room temperature. The cell from a radicle 5 mm. long, shows rather minute granular mitochondria of varying intensity of stain. The protoplasm is distinctly alveolate. No mitochondrial granules occur in the alveoli.

Fig. 11. The plantlet was submerged in water for 24 hours. In the cell figured from the inner cortex of a radicle 9 mm. long, are many enlarged granules and a few short rods varying in intensity of stain and evenly distributed in the protoplasm. There are no filaments in this cell, but in cells of the outer cortex are many lightly stained filaments which are segmenting into shorter rods. The vacuoles contain many intensely stained mitochondrial spheres.

Fig. 12. The plantlet was enclosed in an air-tight space of about 15 c.c. for one day. A cell from the middle cortical layers of a radicle 5 mm. long was selected. The mitochondrial filaments have assumed the form of short rods, spheres and rings which show no tendency to approach the nucleus. They lose their stain entirely in the outermost cells. The vacuoles are large and contain few and sometimes no enlarged mitochondria.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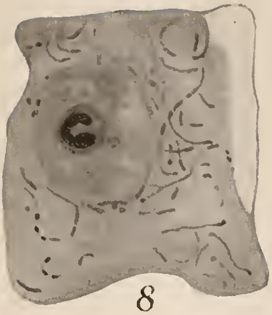
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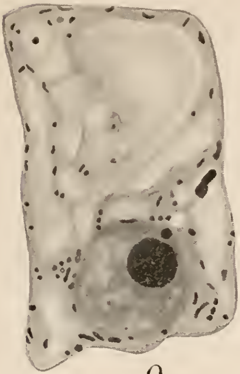
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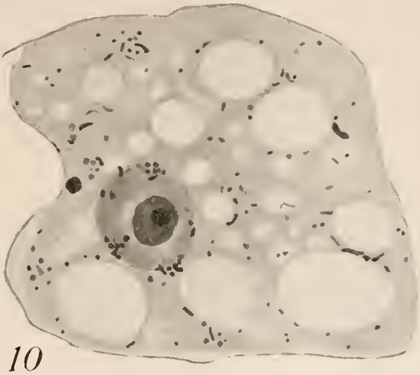
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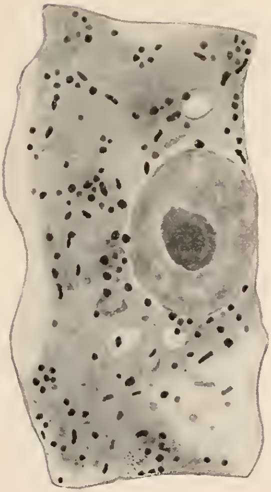
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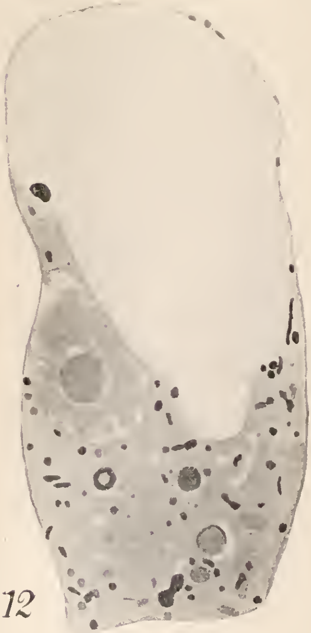
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Plate III.

Fig. 13. The cell figured is from the inner cortex of the same section as Fig. 12. Mitochondria here are almost normal. Filaments are seen with no tendency to approach the nucleus and contrasting strongly with their altered appearance in the middle and other layers.

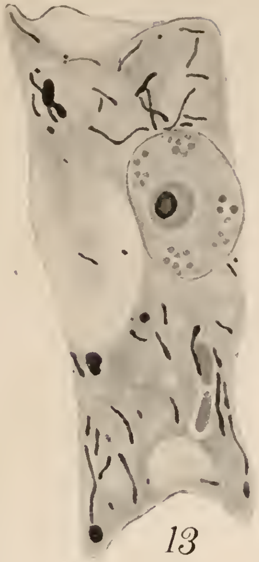
Fig. 14. Plerome cell from a radicle 6 mm. long and exposed to the same confined air-space for two days. Mitochondria have, in many cells, completely disappeared. There are, however, in the cells of the plerome a few lightly stained rods and granules, with some intensely stained, enlarged plast-like bodies. The nucleus shows many strongly stained granules at its periphery.

Fig. 15. Cell from the cortex of a plantlet exposed to the vapor of chloroform, for 2½ minutes in a Petri-dish of 100 c.c. capacity. The radicle is about 7 mm. long. Mitochondria have disappeared leaving no trace except a few lightly stained spherules and dark blotches in the protoplasm. The vacuole is filled with a comparatively darkly stained coagulum.

Fig. 16. Cell from a radicle 10 mm. long exposed to the vapor of ether for 7 minutes under the same conditions as Fig. 15. Mitochondrial granules and rods are very distinct but are irregular in outline. The protoplasm has greatly changed in consistency and appearance.

Fig. 17. The plantlet, grown normally, was placed so that its radicle was immersed in a 10 per cent. solution of glycerine for 18 hours. A radicle 7 mm. long was selected. The cells show a complete disappearance of mitochondria leaving no trace except indistinct darker staining areas in the protoplasm.

Fig. 18. Plantlets grown normally were placed so that their radicles were in contact with a one per cent. solution of lecithin for one day. A radicle 25 mm. long was selected. Mitochondria exhibit a very marked increase in number and size.



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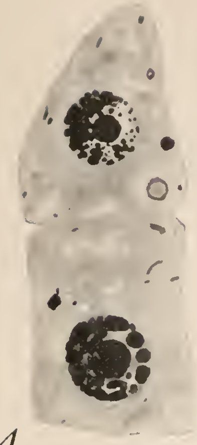

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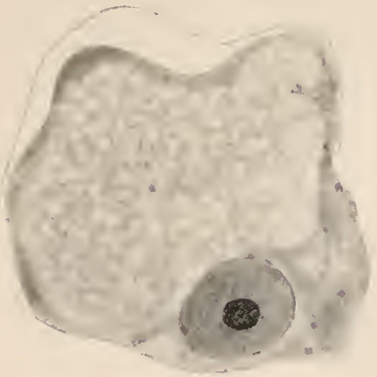

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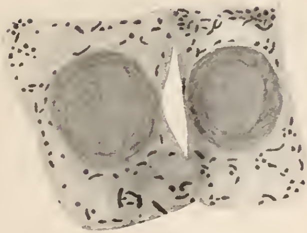

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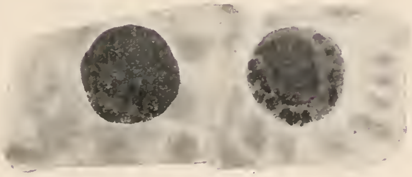

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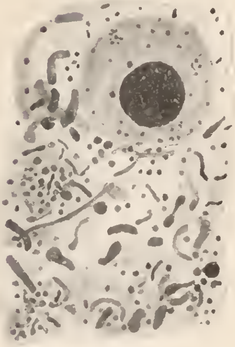

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BIOLOGICAL BULLETIN

THE INTERNAL PHENOMENA OF REPRODUCTION IN DROSOPHILA.

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The present paper is limited to a description of the internal phenomena of reproduction in the vinegar fly *Drosophila melanogaster,* comprising under this title the ejaculation of the sperm by the male, its storage in the seminal receptacles of the female, and the discharge of the spermatozoa from the latter, at the time of the fertilization of the eggs.

The work was carried out in the Marine Biological Laboratory at Woods Hole during the summer of 1919 at the suggestion of Professor T. H. Morgan, to whom I am indebted for advice and helpful criticism, also for valuable information gathered by him in his attempts to bring out artificial fertilization in this fly. An obstacle in the study of the experimental fertilization in *Drosophila* is the high sensitiveness of the spermatozoa to the action of fluids foreign to the body; they are easily injured and killed when placed in mixtures which do not exert noxious effects on the sperm of other animals, unless the spermatozoa are kept in such fluids for a long time. Although I tried repeatedly to eliminate this obstacle by the use of several called "indifferent" fluids, it was impossible to keep the spermatozoa active beyond a few minutes.

During these experiments I gathered interesting data on the normal reproduction of *Drosophila,* in which, owing to the small size and transparent condition of the internal generative organs, it was possible to observe the contents of the extirpated uterus and seminal receptacles of the fertilized fly in the living condition. These organs were kept under direct observation for some time, and the entrance of the spermatozoa into the receptacles and the position of the egg within the uterus were seen directly instead of having to be inferred from series of sections taken at different stages in these processes. Sections have been used as a means of checking up the results obtained by direct observation.

Drosophila has thus provided almost unique material for the study of the internal phenomena preceding normal fertilization in insects, for besides the facts just mentioned, the mating of a considerable number of flies can be easily brought about at any desired time.

1. Technique.

The wild stock of *Drosophila melanogaster* was used throughout the work. Virgin females isolated shortly after hatching were mated to males of the same generation three or four days after isolation. The matings were accurately timed in every case.

The flies were either etherized or killed by a preserving fluid while in copulation or as soon as this was finished. In the first case the observations were made on the living genitalia, these organs being dissected out under a binocular microscope in a drop of Ringer's fluid or normal salt solution, after which they could be observed with a high power microscope. In order to follow the path of the spermatozoa into the receptacles and observe the position of the egg within the uterus, no cover glass was added, and in this way any pressure which might interfere with the first process or crush the egg was avoided. If a cover glass is used and gently pressed down with a needle, the ventral receptacle, normally coiled, will straighten and its contents may be easily observed under high power, the spermatozoa being readily detected when present.

The flies which were preserved were embedded in paraffin and cut into sections. The specimens were always dropped alive in the following fluid, after the formula of W. Docters van Leeuwen:[1]

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[1] *Zool. Anz.*, Bd. 32, 1908.

Picric acid (1 p.c. sol. in abs. alc.)	6 c.c.
Chloroform	1 c.c.
40 per cent. formalin	1 c.c.
Glacial acetic acid	0.5 c.c.

This fluid kills instantly and penetrates very well; but the preservation obtained is poor for cytological purposes yet good enough for histological study. The abdomens of the flies were cut off from the rest of the body while in the fixative, then transferred to 95 per cent. alcohol and cleared with cedar oil, after dehydration with absolute alcohol. Sagittal sections were used almost exclusively and were stained with Delafield's hematoxylin and eosin.

2. The Morphology of the Internal Generative Organs.

The internal generative organs of *Drosophila* are much like those in other flies. In the female they show certain characteristics absent or feebly developed in other diptera.[2]

Male.

The testes (Fig. 1, *t*) are two tightly convoluted tubes of orange or yellow color, which communicate with the vas deferens (*d*) by means of rather short, paired ducts, the vasa efferentia (*v*). The latter are swollen in their initial portion and probably store the ripe spermatozoa before they are discharged into the vas deferens. The vas deferens is a long duct, much swollen in its anterior section, tapering gradually towards its termination at the side of the ejaculatory sac (*s*). Its walls are highly contractile.

The ejaculatory sac is a curious structure which deserves special mention since it plays an important rôle in the ejaculation of the sperm. It acts as a pump and drives the sperm through a narrow ejaculatory duct (*e*) which has stiff walls lined internally with a tough cuticle. The sac can be regarded as an outpocketing of the ejaculatory duct, since it is also lined internally with a cuticle, but its cavity has been cut off from the lumen of the duct which is in communication with the vas deferens through an enlarged portion (Fig. 2, *c*) placed against the proximal surface of the sac. The whole organ may be compared with two kidney-shaped hollow bodies fused along a sagittal plane, their cavities being confluent. The single cavity thus arising is lined with a thin cuticle produced by the columnar epithelium (*w*) which forms the walls of the organ, and contains a highly refractive, thick fluid (*f*) secreted by the epithelium. This fluid is scanty in young flies, a small amount being present in those just hatched. It is entirely separated from the outside since the cavity in which it is contained has no outlet. In the proximal surface of the sac the cuticle secreted by the epithelium is thickened to form a plate bearing a rod-shaped sclerite (*s*) which projects freely into the body cavity. The sclerite is connected with the outer walls of

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[2] So far as is known to the writer, the occurrence of a ventral seminal receptacle has never been mentioned in any of the Diptera. Cf. Berlese, 1909.

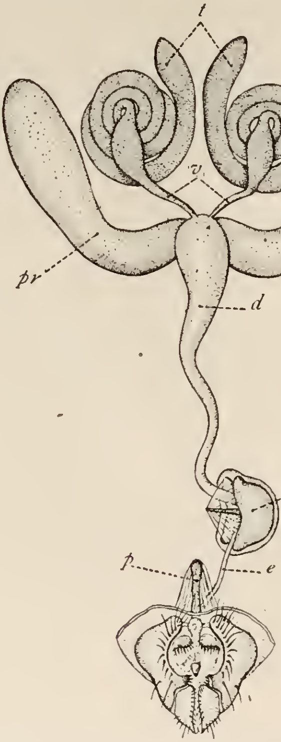

FIG. 1. Male generative organs of *Drosophila melanogaster*, ventral view. *d*, vas deferens; *e*, ejaculatory duct; *p*, penis; *pr*, paragonia; *s*, ejaculatory sac; *t*, testes; *v*, vasa efferentia.

the sac by means of powerful muscle fibers (*m*) which form a cone, the axis being determined by the rod.

The enlarged portion (*c*) connecting the vas deferens with the ejaculatory duct borders the rod-shaped sclerite forming a crescent and therefore is not pierced by it, as inspection of Fig. 2 might lead one to suppose. As the sclerite is a projection from the plate mentioned above, the latter forms a considerable part of the wall of the cavity contained in this enlarged portion, which collects the sperm coming from the vas deferens.

The mechanism that drives the sperm through the narrow ejaculatory duct appears to be very simple but highly efficient.

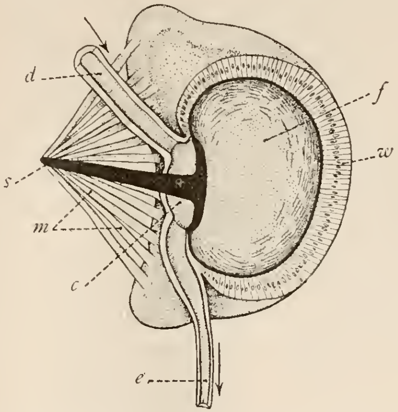

Fig. 2. Diagram of the ejaculatory sac based on the study of sections and on the observation of the fresh organ. The sac has been represented as cut along the sagittal plane. *c*, cavity connecting the vas deferens, *d*, with the ejaculatory duct, *e*; *f*, fluid contained within the sac; *m*, muscle fibers; *s*, sclerite; *w*, epithelial wall of the sac.

Upon contraction of the muscle fibers connecting the sclerite with the walls of the sac, the thick plate at the base of the rod-shaped sclerite sinks into the cavity of the sac, compressing the fluid therein. At the same time the portion connecting the vas deferens with the ejaculatory duct is expanded, the partial vacuum thus produced causing the sperm in the former to flow into its cavity. The relaxation of the muscle fibers aided by the elasticity of the fluid contained in the sac brings the plate of the sclerite to its original position, and as a result the sperm in the cavity is driven out under pressure through the ejaculatory duct.

The return of the sperm into the vas deferens seems to be prevented by the partial or total closure of the orifice of communication.

The thick fluid within the ejaculatory sac is not poured into the duct; in flies killed during copulation or shortly after, no loss of fluid could be observed. On the other hand, the spermatozoa do not penetrate the cavity of the ejaculatory sac, as proved by the study of sections of flies killed during ejaculation. In these the sac appears filled with a coagulated homogeneous fluid, while in the portion of the vas deferens and ejaculatory duct nearest to the organ several bundles of spermatozoa could be seen.

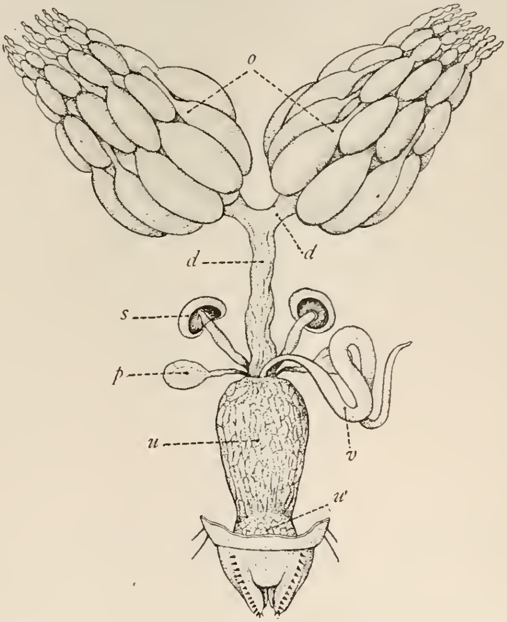

Fig. 3. Female generative organs, ventral view. *d*, oviducts; *d'*, azygos oviduct; *o*, ovaries; *p*, parovaria; *s*, spermathecae; *u*, uterus; *u'*, vaginal portion of the uterus; *v*, ventral receptacle, represented as uncoiled in this figure.

The only accessory glands present in the male are the so-called paragonia (Fig. 1, *pr*), also termed seminal vesicles in spite of the fact that spermatozoa are rarely or never found in them. They are two large sacs which open into the vas deferens, their openings being placed a little below those of the vasa efferentia.

They contain a dense, sticky fluid in which float abundant refractive granules of unequal size. This fluid is mixed with the spermatozoa at their entrance into the vas deferens and forms the liquid part of the ejaculate.

Female.

The ovaries (Fig. 3, *o*) are connected by means of short paired oviducts (*d*) with a single azygos oviduct (*d′*) which opens in the anterior end of the uterus (*u*). The latter is a sac with highly muscular walls, lined internally by an epithelium of cubical cells, which secretes a thin cuticle. Two distinct regions can be recognized in this organ: a distal region or uterus proper (Figs. 3 and 9, *u*) and a proximal region or vagina (*u′*) in which the

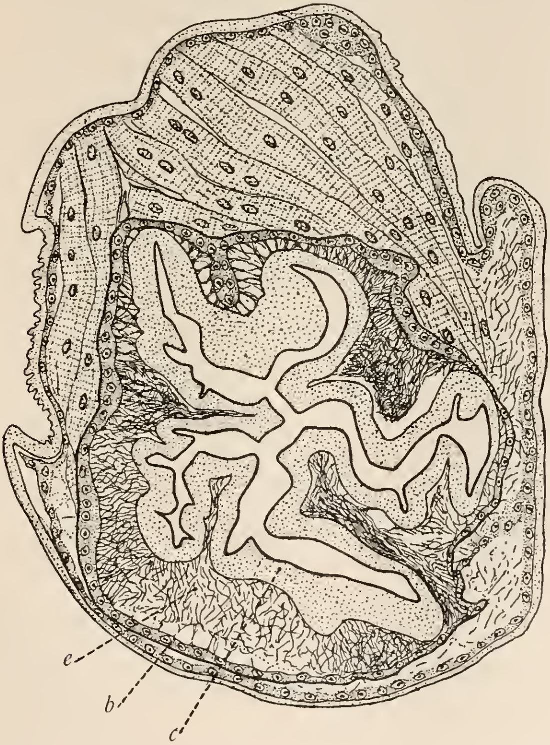

Fig. 4. Cross section of the vagina, showing the irregular lumen and the papillæ in which the epithelium is thrown. *b*, basal portion of the epithelium containing the nuclei; *e*, fibrillar portion; *c*, cuticle.

epithelium is highly modified, the thin cuticular lining being much thicker and thrown into a few very distinct ridges (Fig. 4, *c*). The anterior end of the uterus forms a pouch (Fig. 5, *a*) capable of great distension; its walls are thrown into irregular foldings which in sections appear as papillæ. The oviduct opens dorsad to this pouch by a wide opening (Fig. 5, *d*).

The female of *Drosophila* differs from that of most of the other flies in the presence of an unpaired seminal receptacle placed in the ventral surface of the uterus (Fig. 3, *v*) and by the existence of two smaller receptacles or spermathecæ (*s*). Occasionally three spermathecæ occur and this condition seems to be normal in many of the diptera (Berlese, 1909).

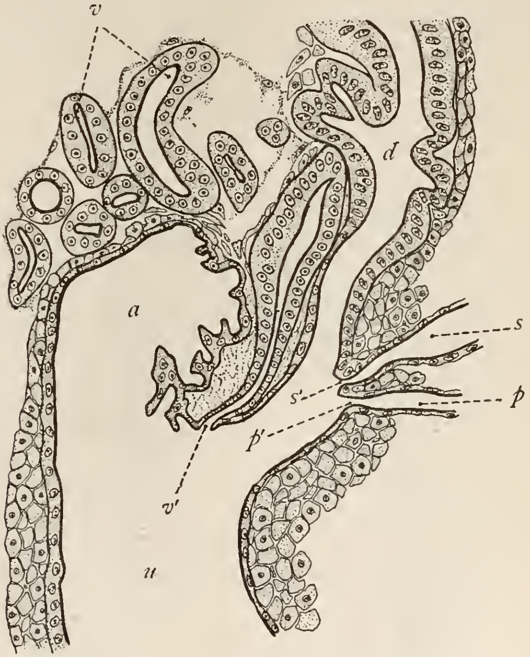

FIG. 5. Sagittal section of the anterior portion of the uterus in a virgin fly. *a*, anterior pouch; *d*, oviduct; *p*, duct of one of the parovaria with *p'*, its opening in the dorsal wall of the uterus; *s*, duct of one of the spermathecæ, with *s'*, its opening into the uterus; *u*, uterine cavity; *v*, ventral receptacle with *v'*, its opening into the uterus.

The spermathecæ (Figs. 3 and 9, *s*) open into the dorsal wall of the anterior portion of the uterus by means of two narrow openings, placed close to each other. They are mushroom-shaped bodies whose terminal cavity is lined by a hard cuticle of brown color (Fig. 6, *c′*) secreted by the epithelium (*e*) forming the walls of the organ. They are connected with the uterus by narrow ducts which on account of circular ridges present in their cuticle closely resemble tracheæ. The circular ridges of the distal portion of each duct bear processes resembling stiff hairs which are directed towards the openings of the ducts (*d*) into the uterus.

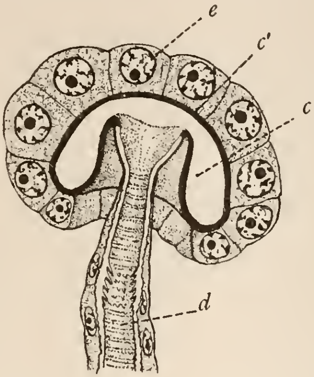

Fig. 6. Diagram of a spermatheca. *c*, cavity lined with the brown cuticle *c′*; *d*, duct of the spermatheca; *e*, epithelium.

The ventral seminal receptacle (Figs. 3 and 9, *v*) is a long convoluted tube which opens into the anterior portion of the uterus, immediately dorsad to the anterior pouch (Fig. 5, *v′*), between the latter and the oviduct. The lumen of this tube is very narrow in its proximal portion, gradually expanding and becoming narrow again in the distal or free portion of the organ. In the normal position the tube is tightly coiled and is placed at the ventral surface of the uterus, immediately above the anterior pouch. The lumen is lined with a cuticle produced by an epithelium of cubical cells.

The size of the ventral receptacle varies a good deal according to the species; in *Drosophila obscura* it is shorter and wide, while in *D. virilis* it attains an enormous length.

A sagittal section of the anterior portion of the uterus (Fig. 5) shows that the opening of the ventral receptacle (*v′*) is placed a little nearer the vagina than the openings of the spermathecæ (s'), and this position seems to have some relation to the entrance of the spermatozoa into the receptacles and their discharge at the time of fertilization.

Besides the organs described there are two other organs (Figs. 3 and 9, p) which correspond in structure with the so-called parovaria in other forms (Lowne, 1893). These organs are obviously glands; they possess a cavity filled with a colloidal fluid containing minute refractive granules. They are connected with the anterior portion of the uterus by means of ducts, which open near the orifices of the spermathecæ (Fig. 5, p'). The walls of the parovaria are made up of large cells which contain round nuclei and a peculiar structure, described in *Calliphora* by Lowne and erroneously interpreted by this observer as a future egg.

In virgin females the wall of the uterus collapses, the lumen being quite narrow and irregular.

3. THE EJACULATION OF THE SPERM AND ITS ENTRANCE INTO THE SEMINAL RECEPTACLES.

The observation of the living uterus and the study of sections of flies killed at different periods during mating showed that the ejaculation of the sperm usually takes place about nine or ten minutes after the beginning of copulation.

Female flies killed immediately after the ejaculation of the sperm showed that, although most of the spermatozoa seemed to be motionless at this time, they occasionally exhibit faint undulatory movements. If they do not move freely it may be because the mass of spermatozoa is subjected to the pressure exerted by the walls of the uterus or possibly to a considerable extent because of the viscid condition of the fluid portion of the ejaculate. But it is evident that they are at least potentially active, as shown by their behavior in later stages.

It was not possible to study the movements of the spermatozoa when outside of the uterus, the fluids foreign to the body causing at first a decrease in their activity, followed by their death. I have dissected flies in several fluids such as tap and distilled water, normal saline solution (0.5 per cent.), Ringer's fluid (coldblooded), 1 per cent. solution of potassium citrate,[1] and 1 per thousand solution of sodium hydroxide. All of these fluids injure the spermatozoa more or less rapidly, and fail to keep them alive beyond two or three minutes. On acount of the small size of the flies it was not possible to obtain body plasma in sufficient amount to tease the uterus in it and observe the behavior of the spermatozoa.

The spermatozoa do not enter the seminal receptacles immediately after ejaculation has taken place. There is a pause which usually lasts two or three minutes or even more, during which the cavity of the uterus is packed with spermatozoa while the seminal receptacles are entirely empty. This peculiarity was observed once, and again in the uterus of flies killed after ten or eleven minutes of copulation. These results are consistent with those obtained by the study of sections of flies preserved at the same stage, the spermatozoa being crowded in the uterine cavity, while the receptacles and their ducts do not show any spermatozoön whatsoever.

The occurrence of this pause suggests that some change must take place that causes the spermatozoa to swim actively, for the swimming movements are very conspicuous when they enter the receptacles in contrast to their almost passive condition while in the uterus. This fact points to an influence on the part of the parovaria which are the only accessory glands in the female genitalia whose function is obscure. Their bearing on the formation of the shell of the egg is very doubtful, the latter being completely formed when entering the uterus. Lowne thought that the parovaria in the blow-fly furnish the very young oöcytes; he reached this striking conclusion on account of the presence of certain structures in the cytoplasm of their cells which he compared with the oöcyte of mammals since they are surrounded with a thin layer resembling the zona pellucida of the mammalian egg. As already mentioned these curious structures are also present in the parovaria of *Drosophila*. Lowne's interpretation is not warranted by any fact, other than a superficial resemblance, the two structures being essentially of a different kind. It seems unnecessary to insist here upon this point. The glandular nature of the parovaria is the more probable interpretation.

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[1] According to Koltzoff, 1908, the spermatozoa of the rhinoceros beetle, *Oryctes nasicornis*, may be kept alive in a 2 per cent. aqueous solution of potassium citrate for a week; this observer thinks that their death is caused by the growth of bacteria in the fluid rather than through exhaustion.

However, it was not possible to ascertain the kind of influence exerted by these glands on the spermatozoa, since all the experiments to solve this problem had to be carried out in a fluid foreign to the body, which, as stated above, kills the spermatozoa. Under such conditions it is hardly possible that the secretion of the parovaria may cause any response in already injured elements. While the secretion of the parovaria may activate the spermatozoa it is still possible that they are concerned only with the dissolution of the thick fluid portion of the ejaculate, thus removing one of the obstacles which prevent free motion.

The entrance of the spermatozoa into the receptacles has been repeatedly observed by the writer in the living genitalia, for several minutes at a time. Owing to the impermeability of the walls of the receptacles and their ducts, the noxious effects of the fluids in which the dissections were carried out were not felt at once. The ventral receptacle is the first to receive the sperm. Since the amount of sperm ejaculated usually exceeds the quantity which can be stored in the receptacles it is common to see spermatozoa which have made their way into the oviduct. As will be pointed out later, on account of the position of the egg when passing through the latter, it is safe to assume that such spermatozoa do not play any rôle in the fertilization of the first eggs coming into the uterus, which very often, and for reasons not well known, are sterile.

At the time of their entrance into the ventral receptacle, the spermatozoa in the vicinity of the openings of the parovaria, namely, those crowded in the anterior portion of the uterus, begin to show strong movements and make their way into the receptacle by swimming actively, a stream of them being detected along the coils of this organ. When they reach the tip of the latter the swimming movements cease, to be replaced by undulatory movements, which decrease in intensity as new spermatozoa arrive. After two to five minutes the whole lumen of the receptacle is packed with sperms.

When the ventral receptacle is filled with sperms, the same process is repeated in the spermathecæ. Here it is still easier to follow their entrance, for the ducts of these receptacles are very thin and transparent. In no case were spermatozoa observed entering these receptacles before the ventral receptacle was filled with spermatozoa. This might be explained by the position of the orifice leading to the latter, which, as already stated, is placed a little posterior to the openings of the spermathecæ, and perhaps also by the partial closure of the orifices leading to these organs.

The spermatozoa contained in the seminal receptacles form bundles; in the ventral receptacle all the heads are directed toward the tip or free end of the organ. In the spermathecæ the bundles are concentric.

Once within the receptacles the spermatozoa cease to swim, showing only undulatory movements; in this condition they can live for many days, even weeks.

5. The Egg and its Entrance into the Uterus.

The eggs of *Drosophila melanogaster* are very small, elliptical in shape and bear at one end a pair of diverging appendages

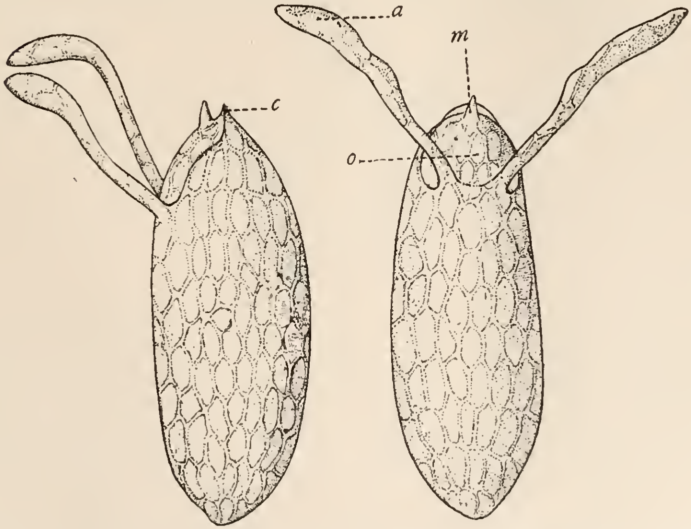

Fig. 7. Dorsal and lateral views of the egg of *D. melanogaster*. *a*, appendages; *c*, plate formed by the edge of *o*, transparent area resembling an operculum; *m*, micropyle cone.

whose function is not well known. They probably prevent the sinking of the egg when laid on the fermented fruit, thus allowing the hatching larvæ to creep over the food without being asphyxiated. These appendages (Fig. 7, *a*) arise from one of the surfaces of the egg. For descriptive purposes I will call it the dorsal surface since it is applied against the dorsal wall of the uterus when the egg is within the latter.

The end of the egg nearest to the place from which the appendages arise bears a conical structure, the micropyle cone (*m*), which is pierced by an extremely narrow canal, the micropyle. Observed under higher power, the micropyle cone appears hollow (Fig. 8, *c*) with the cytoplasm of the egg (*p*) penetrating its interior. The canal or micropyle (*m*) goes from the tip of the cone towards its base and is placed on the side continuous with the ventral surface of the egg. The pole of the egg bearing the micropyle is always directed toward the anterior end of the animal, either when in the ovary (Fig. 3) or when in the uterus (Fig. 9).

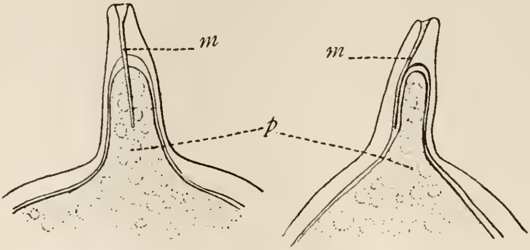

FIG. 8. Ventral and lateral views of the micropyle cone of *D. melanogaster* drawn from the fresh egg. *m*, micropyle; *p*, protoplasm of the egg penetrating into the micropyle cone.

In the portion of the dorsal surface in front of the appendages there is an area more transparent than the rest of the chorion, which could be termed the operculum in the event of the escape of the hatching larva through this region of the egg. However, this does not always happen, the anterior end of the egg splitting rather irregularly and further ventrally than this.[1] The lateral borders of the transparent area form a ridge which appears as a thin plate at the apex of the egg, passing ventrally the micropyle cone.

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[1] I am indebted for this observation to Dr. A. H. Sturtevant.

The outer chorion of the egg shows hexagonal sculpturing produced by the hardening of the much flattened follicle cells surrounding the oöcyte during the growth period. This pattern is a little less developed in the transparent region described above (Fig. 7, *o*).

The passage of the eggs through the oviduct takes place very quickly if we judge from the considerable number of egg-laying females dissected in which no eggs were present in this duct. The actual entrance of an egg into the oviduct was observed only

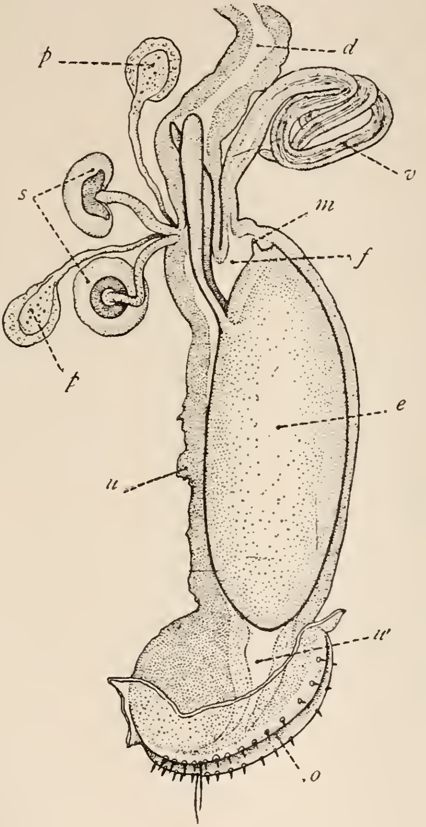

FIG. 9. Lateral view of the uterus containing an egg; this figure was outlined with the camera lucida, the genitalia being in the fresh condition. *e*, egg with the appendages within the oviduct, *d*; *f*, fertilization cavity; *m*, micropyle cone of the egg; *o*, vaginal orifice; *p*, parovaria; *s*, spermathecæ; *u*, uterus with *u'*, its vaginal portion; *v*, ventral receptacle, containing spermatozoa.

once. Waves of contraction were noticeable in one of the ovaries, extending from its tip to the base and caused apparently by the contraction of muscle fibers placed in the thin connective tissue layer enveloping the organ. At the same time the oviduct expanded and contracted rhythmically thus bringing one of the eggs lying near the entrance of one of the paired oviducts into its lumen. The contractions were strong enough to push the egg into the oviduct, and probably the process would have gone further had it not been for the exhaustion brought about by the artificial environment in which the organs were placed. These contractions were very conspicuous in the ovaries of all the flies dissected, whether laying eggs or not, and may be explained by the action of the fluid in which dissection was carried out, or by the action of free oxygen on the muscle fibers of the ovary and oviduct, which take the place of the nervous stimulus in the living fly.

The orientation of the egg, when passing through the oviduct, is the same as that in the ovary, the micropyle cone being directed toward the latter. The egg is about three times as wide as the oviduct, which is, therefore, considerably distended when the egg is passing through it.

These conditions make impossible the fertilization of the eggs in the oviduct. Since the micropyle is not facing the opening of the latter into the uterus, and since the egg is larger than the oviduct, any spermatozoa, if present, would be pressed aside by the passing egg in the event of their penetration into the oviduct.

The size of the uterus is so well adapted to that of the egg that the latter occupies almost entirely its cavity (Fig. 9), the appendages remaining within the oviduct (*d*), thus blocking the way of any spermatozoa that might enter into the latter when the sperm is poured out of the seminal receptacles during fertilization. A small space (*f*) is left in the anterior portion of the uterus, between the walls of the anterior pouch and the surface of the egg, and in this space swim the spermatozoa coming from the seminal receptacles.

The position of the egg within the uterus is constantly the same in all the flies observed, including several females of *Drosophila obscura*. The dorsal surface of the egg, where the appendages arise, is applied against the dorsal wall of the uterus, while the opposite surface lies against the ventral wall. The anterior end of the egg, bearing the micropyle is lodged in the anterior pouch of the uterus; the micropyle cone is directed toward the opening of the ventral receptacle, and in some cases seems to be almost in contact with it.

Owing to the size of the egg no space is left between its chorion and the wall of the uterus; both of these come in close contact thus preventing the spermatozoa from being diverted from the anterior pole of the egg, where the penetration of a spermatozoön takes place.

6. The External Phenomena of Fertilization.

Under this heading I will consider all the phenomena leading to the penetration of the egg by a spermatozoön, the discharge of the sperm from the ventral receptacle and spermathecæ, and the way in which the last process is accomplished. The actual penetration of the egg by the spermatozoön was never observed owing to the lack of transparency in the walls of the anterior portion of the uterus, which prevents the detection of isolated spermatozoa, and also to the fact that it is very difficult to ascertain the moment in which an egg enters into the cavity of the uterus.

A series of experiments showed that in all probability the first spermatozoa used in fertilization are those stored in the ventral receptacle, those kept in the spermathecæ being used later, when the contents of the former are exhausted. When planning these experiments it was thought that the best way to ascertain the facts would be to allow several females to be fertilized and examine them at definite periods after which the condition of the ventral receptacle and spermathecæ could be observed by dissection. The time chosen for the first lot of flies was seven days. The food was changed daily, and the eggs laid counted every day. There is, however, an important obstacle, namely, that the rate of laying is variable in different females. But this proved to be very helpful, for, instead of the ventral receptacle being found empty in all the flies, a rough relation between the number of eggs laid and the amount of sperm stored in this receptacle was found. Ten flies were used in this experiment; two of them died in the first four days, the others laying eggs continuously during the seven days. The flies belonged to the same generation, were isolated on the same day, the isolation period prior to mating being three days. The number of eggs layed was 470, 336, 335, 238, 221, 206, 200 and 117. In the female which laid 470 eggs only a few spermatozoa could be detected in the ventral receptacle. In the female which laid the smallest number of eggs (117) about two thirds of the ventral receptacle was filled with spermatozoa. The amount of sperm present in the ventral receptacle of the other flies could not be estimated accurately, but decreased noticeably as the number of eggs laid increased. Inspection of the spermathecæ in all of the eight flies showed the presence of abundant spermatozoa closely packed; no spermatozoa were detected along the ducts of these organs. Additional dissections of flies killed from twelve to fourteen days after fertilized proved in all cases the correctness of this view.

As the ventral receptacle begins to empty its spermatozoa it may be seen that the latter are turning around so as to have their heads directed toward the opening of the receptacle into the uterus. This rotation was very conspicuous in most of the flies dissected, the spermatozoa being scattered in the proximal portion of the receptacle without definite orientation.

The bulk of the sperm does not go to the immediate vicinity of the opening of the ventral receptacle into the uterus when their discharge begins, but remains in the free end of the receptacle, only a few traversing the empty space which separates the latter from the orifice, to be poured into the anterior portion of the uterus. The inspection of the initial portion of the receptacle showed a few spermatozoa swimming towards the uterus when this contains an egg; the portion of the receptacle near the opening is so narrow that only a few spermatozoa can pass at a time.

Owing to the position of the micropyle in the egg within the uterus it was thought that the spermatozoa stored in the spermathecæ might be unable to fertilize any eggs, since the orifices leading to these organs are placed in the dorsal wall of the uterus and the appendages of the egg come to lie between these orifices and the micropyle. Some experiments were carried out in order to ascertain this important point. Ten flies were simultaneously mated to males of the same generation, then isolated in vials, the food being changed daily. When the first sterile eggs were laid by each fly it was dissected and the conditions of the spermathecæ carefully observed. The following table shows the result of this experiment, in which the number of eggs laid each day was not recorded.

Table I.

Flies.	No. of Days Laying.	Condition of the Spermathecæ.	Condition of the Ventral Receptacle.
1	20	Sperm present	Empty
2	12	Empty	"
3	22	"	"
4	21	"	"
5	20	"	"
6	22	"	"
7	20	"	"
8	20	"	"
9	20	"	Sperm present
10	22	"	Empty

With the exception of fly No. 1, which laid some sterile eggs while the spermathecæ still retained spermatozoa, all the other flies showed no spermatozoa at all. Whether the sterile eggs laid by such a fly had received spermatozoa or not is a point impossible to settle. It is possible that the sterility in this case was related to intrinsic factors in the egg, rather than to a failure in the discharge of the spermatozoa from the receptacles. The ventral receptacle appeared empty in all of the flies except specimen No. 9 which showed a quite irregular behavior in the rate of laying. I do not know the causes of the retention of spermatozoa in the ventral receptacle of this fly, which appeared entirely normal in other respects.

These results show that all the spermatozoa, whether they are stored in the ventral receptacle or in the spermathecæ, are used in fertilization. The presence of foldings in the epithelium lining the anterior pouch of the uterus probably plays an important rôle in the fertilization of the eggs by spermatozoa coming from the spermathecæ, since such outgrowths of the epithelium form deep grooves along which the spermatozoa may progress until they reach the empty space left between the egg and the walls of the uterus.

Although, as stated above, the presence of sperm in the spermathecæ of flies which have been laying eggs for several days suggests that the first spermatozoa used in fertilization are those stored in the ventral receptacle, which appears empty or containing few spermatozoa, still the same conditions may appear if the amount of sperm received by the spermathecæ surpasses by far that stored in the ventral receptacle. In other words, the largest amount of sperm will last longer, provided that spermatozoa are simultaneously set free by all the seminal receptacles.

Further experiments were carried out with the hope of clearing up this point. These experiments consisted in allowing fertilized females to lay eggs during a period of six days, and then cross them with a mutant which would enable me to recognize the hybrids in the F_1, without need of testing the offspring. The mutant used was the bar-eyed male. Seven flies were used in the experiment, which covered a period of eighteen days. The food was changed daily and placed in separate vials to allow the eggs to develop.

These experiments showed the following facts: (1) When the number of flies produced during the first six days is low, the offspring following fertilization by the bar male consists of hybrid and normal females, the males being normal in all the cases. This result can be accounted for by the presence of spermatozoa from the first copulation in the ventral receptacle where they become mixed up with those received during copulation with the bar male. (2) When, on the contrary, the number of flies produced during the first six days is high, only hybrid females are produced during the first days following fertilization by the bar male. That is to say, the ventral receptacle was already empty of sperm. It was then filled by the sperm ejaculated by the bar male, and, therefore, only hybrid females were produced. In such a case mixed offspring appear after a few days, when the spermatozoa stored in the spermathecæ begin to be used.

The results obtained in the second case have been graphically shown in the curve of Fig. 10, plotted after the data obtained in a female which had produced a large number of flies during the

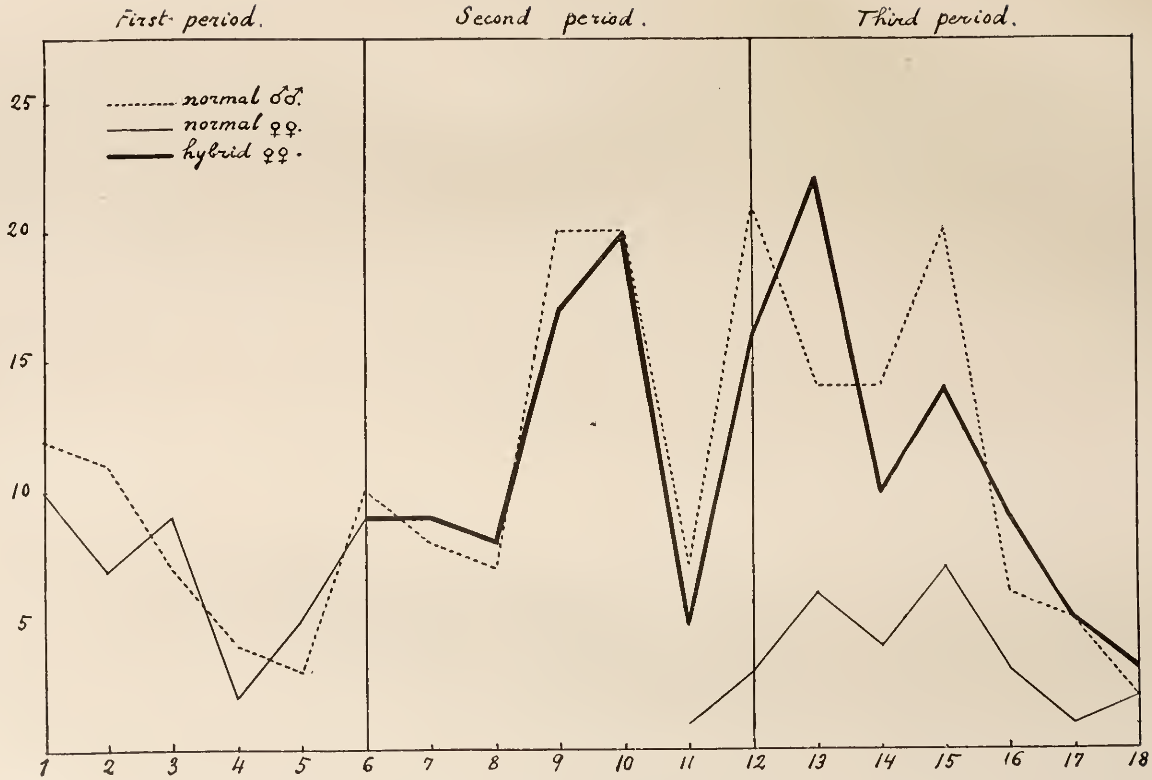

FIG. 10. Curve representing the results of crossing a normal, fertilized female with a male of the bar-eyed mutant. During the second period only hybrid females are produced, showing that the spermatozoa kept in the ventral receptacle at that time are those from the bar male.

first period.[1] During the first five days after mating to the bar-eyed male only hybrid females were produced; at the sixth day normal females began to appear. Fortunately this fly was killed at a critical moment, when fertilization of the eggs was about to stop. Dissection showed that the ventral receptacle and one of the spermathecæ were empty, while a few active spermatozoa could be seen moving along the duct of the other spermatheca.

It is interesting to note that the number of normal females produced after crossing with the bar male is smaller than that of the hybrid females. This peculiarity, which stands out clearly in all the experiments, is due to the fact that the number of flies hatched is higher when the parents belong to different mutant races.

An important point in the mechanism of the discharge of the sperm from the seminal receptacles is the influence exerted by the egg on the spermatozoa. Whether or not the latter are attracted by the egg is a point which could not be determined by the writer. Although such an attraction, due to a chemotactic stimulus, is possible, we must keep in mind that the same result may be attained by reflexes set up when the egg enters the uterus, the release of the spermatozoa taking place upon dilatation of the orifices leading to the seminal receptacles. The behavior of the spermatozoa in sterile flies would throw some light on this problem, showing at least whether their discharge is related to the entrance of an egg into the uterus. Such sterile flies are said to be rather common in the inbred stocks of *Drosophila melanogaster* (Hyde, '14); they do not lay eggs, probably on account of the abnormal condition of their oviducts. If there is any relation between the laying of eggs and the discharge of the spermatozoa, such flies not laying any should keep the spermatozoa in their receptacles; otherwise the latter would appear empty after some time. Some sterile flies which appeared in the bar-eyed stock were given to me by Dr. Morgan, in the hope that this point could be settled. Unfortunately such flies could not be mated, the males giving up courtship after a short time. Very often a male

[1] The number of flies hatched must not be taken as a fair representation of the number of eggs laid. Both males and females may die in relatively large numbers during development, either as the result of intrinsic lethal factors or of inappropriate environmental conditions. To this is added the sterility of some eggs, especially during the first days following copulation.

which would readily mate, having been kept isolated in a vial for three or four days, becomes discouraged after smelling the posterior end of the female, where normally a white matter acting as a cause of sexual excitement protrudes (Sturtevant, '15). Sterile flies usually die after a few days, their abdomens attaining considerable distension. In the only female dissected the paired oviducts lacked a lumen; some four or five eggs had fallen into the body cavity by rupture of the ovarian wall, and were entirely degenerated, the cytoplasm appearing in irregular clumps. All the seminal receptacles, which like the uterus were normal, did not contain sperm.

Conclusions.

1. In the internal genitalia of *Drosophila melanogaster* there are only two spermathecæ and a ventral seminal receptacle in the form of a long, convoluted tube which opens proximally into the anterior portion of the uterus, ventral to the oviduct.

2. The spermatozoa after ejaculation into the uterus during copulation appear almost motionless, only a few showing faint undulatory movements. The ejaculation of the sperm by the male is greatly aided through the action of the ejaculatory sac, an organ which acts as a pump, driving the sperm at a high pressure through the narrow ejaculatory duct.

3. After ejaculation of the sperm there is a pause which lasts two or three minutes, during which the spermatozoa do not show active movements. Then they begin to swim actively to enter the seminal receptacles. There seems to be an activation of the spermatozoa at this moment on the part of the parovaria, two glandular structures connected with the anterior portion of the uterus by means of narrow ducts.

4. The ventral receptacle is the first to receive the sperm, which is also stored in the spermathecæ. The spermatozoa form bundles when in the receptacles. In the ventral receptacle all the heads are directed toward the distal end of this organ. The bundles are concentric in the spermathecæ.

5. The position of the egg within the uterus is constant; the surface bearing a pair of divergent appendages is applied against the dorsal wall of the uterus. The anterior end of the egg with the micropyle is lodged in the anterior portion of the uterus, with

the micropyle cone directed toward the opening of the ventral receptacle. The appendages of the egg remain within the oviduct thus preventing the entrance of spermatozoa into this organ. Fertilization of the eggs takes place in the uterus.

6. The observation of the extirpated internal genitalia in fertilized flies which had been laying eggs for several days and some experiments show that in all probability the first spermatozoa used in fertilization are those stored in the ventral receptacle, while the spermatozoa kept in the spermathecæ are used later.

7. The influence of the secretion of the parovaria on the spermatozoa and the behavior of the latter when in the vicinity of the egg could not be ascertained on account of the injurious effects of the fluids in which these experiments were carried, on the spermatozoa, which are very sensitive to fluids foreign to the body.

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THE EFFECTS OF CASTRATION OF HEN-FEATHERED CAMPINES.

T. H. MORGAN.

In the Sebright Bantam I have shown (1913, '15, '17, '19, '20) that castration changes the hen-feathered male into a cock-feathered bird. There is only one type of male in the Sebright race—the hen-feathered bird. In a few other races of poultry, two kinds of males are known, one hen-feathered, the other cock-feathered. Both are fertile, and sometimes one type is recognized as the standard, at other times and places the other type. It is very probable, from evidence that will be given later, that the hen-feathered Campine type is dominant over the cock-feathered. This explains why it is more difficult to find a race pure for hen-feathering; because both the males and the females of such a race may carry the recessive factor for cock-feathering. It is known that many of the stocks, supposed to be hen-feathered, occasionally "throw" cock-feathered males. Now that the cause of this "reversion" is understood, it should not be difficult to produce a race pure for hen-feathering. In fact, even the ordinary way of breeding, if combined with a strict selection, would in time bring about such a result.

For several years I have been desirous of performing on such a dimorphic race as the Campines the same castration experiment that had been successful in the Sebrights; but it was essential to find first a stock that was pure for hen-feathering. Fortunately the stock of Mr. Martling of Ridgefield, N. J., appears to satisfy this condition. I have seen the birds in his flock for three years, and in one year all of the juvenile cockerels before any selection amongst them had been made. They were all hen-feathered with the rare exception of a male that had "broken," in the sense that a few feathers were changed in the direction of cock-feathering. The history of one such bird, to be described below, shows that the occurrence of these "breaks" does not prove the impurity of such stocks. The statement of Mr.

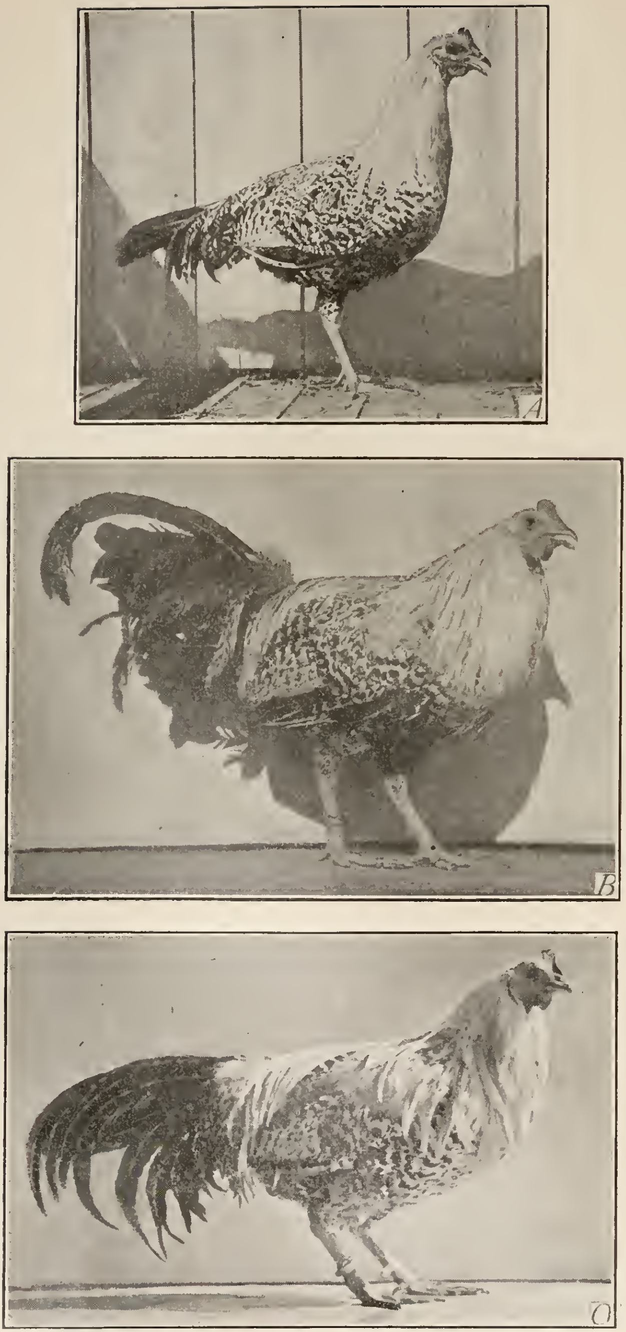


FIG. 1. Youngest bird (castrated June 16); *A*, as it appeared on Sept. 9; *B*, on Nov. 12, 1919; and *C* on Apr. 16, 1920.

Martling that such birds later become hen-feathered is further and important evidence on this point.

In May, 1919, I obtained three young cockerels that had hatched about the first of March, 1919. The youngest had hatched a week later than the other two, and was still in its juvenile plumage, although feathers of the next plumage were just beginning to push out. The bird still peeped. The other, larger birds had just begun to crow and had some of their secondary plumage fairly well developed in critical regions of the body (back, saddle), and this showed definitely the hen-feathered condition. These two birds were castrated on June 16, 1919. The testes of both were in the juvenile condition, and were readily removed, although a piece of one testis of the larger bird broke off and its complete removal was indicated as doubtful. Typical feathers from each of these birds are shown in Fig. 2, *a, b, c, d,* Fig. 5, *a, b, c, d.* As the new feathers came in during the following weeks and months they showed clearly the effects of castration; that is, they were cock-feathered or strongly marked in that direction. The younger bird had just begun to develop its second plumage, and as the feathers continued to appear, the first of them were hen-feathered in pattern and in shape at the tip of the feather, while the base only was affected by the castration and showed some of the characters of cock-feathering. These results go to show that the young bird also would un-

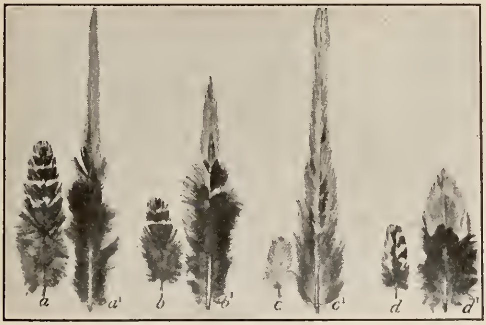


FIG. 2. Feathers of same bird (Fig. 1); *a–d*, before, and a^1–d^1, after castration, Nov. 13.

doubtedly have been hen-feathered if it had not been castrated. Further details concerning these two birds are seen in the photographs of the birds and of their feathers. The appearance of the castrated "younger" bird on September 9, may be gathered from Fig. 1, *A*.

He was obviously becoming cock-feathered at this time, but the change was not as great as it became later. The juvenile feathers present on June 16 (at the time of operation,) are shown in Fig. 2, *a, b, c, d*. The changes that had taken place by November 12, are shown in the photograph of the bird (Fig. 1, *B*) and in the pictures of the feathers (Fig. 2, a^1, b^1, c^1, d^1.) The last picture of this bird was taken April 16, 1920 (Fig. 1, *C*). The picture shows the long, white neck-feathers, that are almost white. The long, white saddle feathers are also well shown in the photograph. The tail coverts and the sickle feathers are very long and the upper ones are black. Later the sickles became even longer. The white shoulder feathers can be seen through the neck hackles. The details of the feathers are better shown in

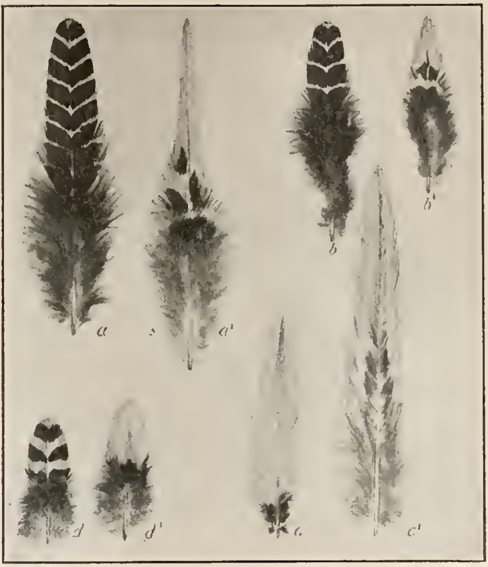
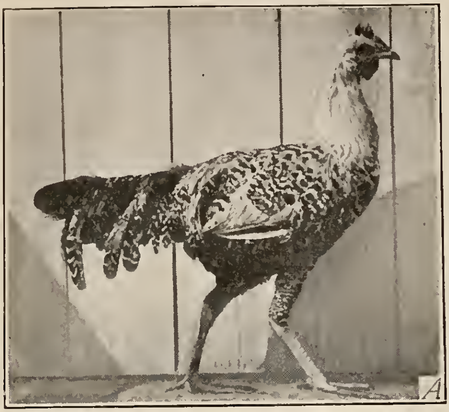


FIG. 3. *a, b, c, d.* feathers of adult hen-feathered Campine (Fig. 8, *A*), and a^1, b^1, c^1, d^1, those of Fig. 1, *C*, March 13.

Fig. 3, a^1, b^1, c^1, d^1, where they are accompanied by feathers from the same region of an adult hen-feathered Campine (Fig. 3, *a, b, c, d*). Taking these in pairs we see that the beautiful barring of the saddle feather (Fig. 3, *a*) gives way in the castrated bird to a pointed white feather, black only at its base (Fig. 3, a^1). The sides of the outer portions of this feather lack barbules as in the common cock. The feathers of the back of the hen-



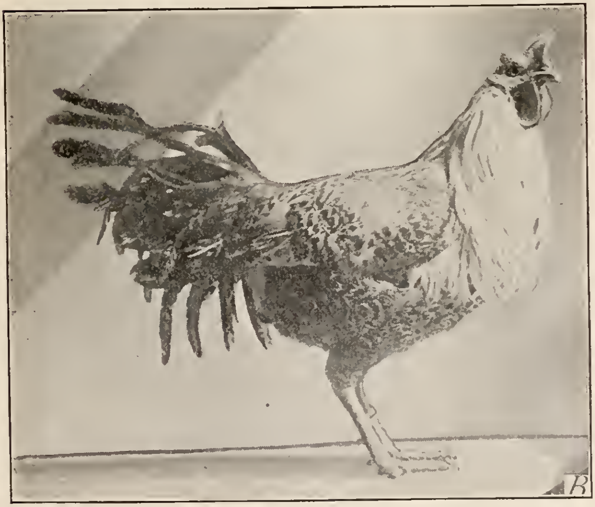


FIG. 4. Second "larger" bird castrated June 16, 1919. *A*, as it appeared Sept. 9; and *B*, as it appeared March 2, 1920.

feathered male are also barred; this too is lost after castration (Fig. 3, *b*, b^1), when the tip becomes white, more pointed, and hackled. The change in the neck feathers (Fig. 3, *c*, c^1) is not so striking because they are already somewhat elongated and white as in the hen, but after castration they increase greatly in length, more in fact than shown by the feathers in the pictures.

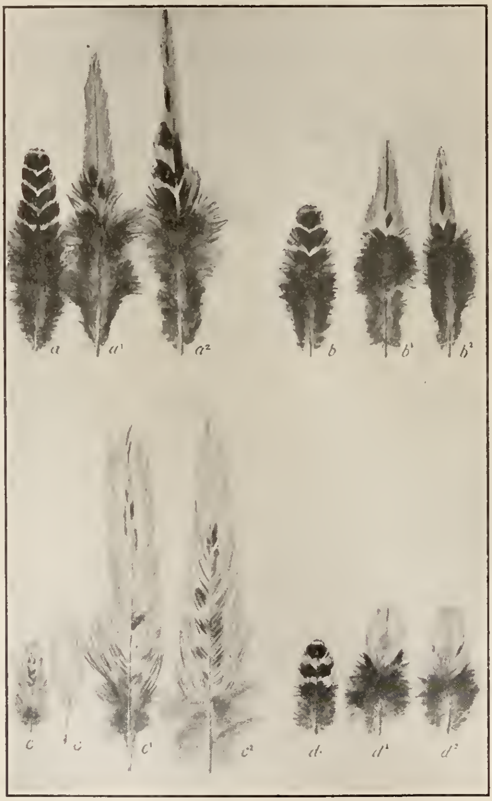


FIG. 5. Feathers of Fig. 4, *a*, *b*, *c*, *d*, before castration, and a^1, b^1, c^1, d^1, after castration on Sept. 15, and a^2, b^2, c^2, d^2, on March 3, 1920.

The feathers of the shoulder (wing-bow), of the hen-feathered male are barred with broad bars, Fig. 3, *d*. After castration the barring is lost, the outer ends of the feathers become pure white, and on each side barbules are lacking over a broad zone. This change, also, is towards cock-feathering as seen by comparing

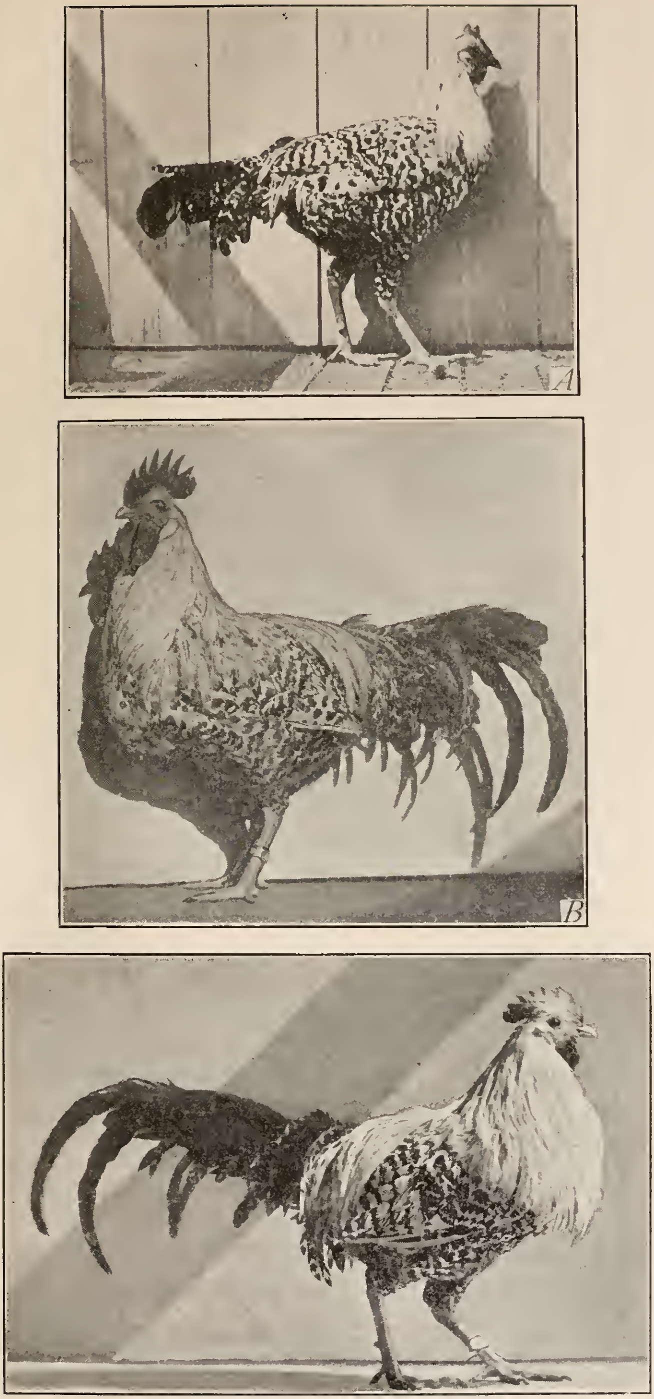


FIG. 6. *A*, third Campine that showed in its first adult feathers indications of cock-feathering, Sept. 9. Castrated Sept. 13. *B*, as it appeared Nov. 12; and *C*, as it appeared on March 6, 1920.

Fig. 1, *C*, with that of the cock-feathered Campine in Fig. 9. The bird was killed May 9, 1920. On the right side in the position of the testis there was found a very small piece of testicle-like tissue. In addition there were two sacks filled with black matter, attached loosely to the mesentery near the old position of the testis.

In this "small" Campine a piece of testis was found, as stated, in the situs of the removed testis. The histological structure of this regenerating testis is entirely similar to that in the regenerating piece of the Campine that was castrated when cock-feathers were beginning to appear. The description of the latter may serve for that found in the small Campine; both pieces agree in all details: the lutear cells have disappeared entirely and large numbers of small lymphocytes are produced in the mesenchyme. The spermatogenetic process is normal in the tubules that had been left, but the spermatozoa are degenerate. New seminal tubules are arising, but they are reduced to the Sertoli cells, no spermatogonia being present.

Besides this piece of regenerating testis there were found dark masses of tissue. In these masses there has been an active proliferation of connective tissue cells, which form strands placed in all directions. Some of the cells in this stroma are filled with large drops of a fat-like substance. There is an active hematopoiesis in these masses, the large lymphocytes being abundant, both within and outside the blood vessels. Large accumulations of normal and degenerated erythrocytes occur in the blood vessels, hence the dark color of the masses. The aspect of the latter suggests that they are portions of the degenerated epydidimis, a few tubules of the latter being present, although their epithelium is almost entirely degenerated.

The extent to which the second "larger" bird had changed by Sept. 9, is shown in Fig. 4, *A*. His new back and saddle feathers were long and pointed. He continued to become more cock-feathered, but his comb began to grow, and it became evident that some of the testis had been left. He was opened under ether. Attempts were made to remove the pieces, but the operation was not successful as shown by the condition of the comb which remained large. It was however very pale. The bird was in such poor condition after the second operation that for a time it was expected that he would die, but he finally got better and lived several months. When the bird was killed a large growth of bony substance was found on the left femur that fitted into a deep depression in the ribs. Some sort of infection had occurred at the region of incision. The photograph shown in Fig. 4, *B*, was taken March 2, 1920. The bird was at this time completely cock-feathered as best seen in the feather chart Fig. 5. Here three feathers from each region are shown. The first (*a, b, c, d*) are those present on June 16, when the first operation was performed. They are typical for a hen-feathered bird of this age. The change that took place before the second operation can be seen from inspection of the second set of feathers (a^1, b^1, c^1, d^1, Sept. 15). The third set of feathers (a^2, b^2, c^2, d^2), pulled out March 3, 1920, show that no further change had taken place; in fact there is some evidence of slight retrogression, which is sufficiently accounted for by the continued growth of the pieces of the testis that had been left. When killed on May 8, there were found three small sacks filled with black material on the right side, and one good but small piece of testis on the right side somewhat larger than a pea.

The third bird had been picked out of the flock, because its secondary plumage, that was just coming in, showed on the back and saddle, marked indications of cock-feathering (Fig. 6 *A*). It was obviously an exceptional bird. It was not operated on when the other two were. During June and July, as new feathers came in, this bird became more markedly cock-feathered, as seen in the photograph taken Sept. 9 (Fig. 6, *A*). Nevertheless none of the feathers were as fully developed as would be expected in a normal cock-feathered male of this race, as seen by comparing Figs. 5 and 7. The comb of this bird remained small at first, but slowly got larger. It was much paler than the comb of the normal hen-feathered males of its race and not quite so large. On Sept. 13 the bird was etherized and opened at the side between the last pair of ribs as in the ordinary process of castration. Both testes in this bird were little advanced beyond the juvenile stage, and were not nearly as large as expected in a bird of this age and size. In this condition we find, I think, an explanation of the appearance of cock-feathers. Something had kept back the normal growth of the testes, and their small size was insufficient to entirely suppress the cock-feathering that the genetic complex of the bird called for. The testes were sectioned and studied by Dr. José Nonidez who furnishes the following report. "The luteal cells are very abundant in the spaces between the tubules especially in the periphery of the testes. In the clusters of luteal cells, however, most of them appear to be undergoing degeneration, for, the nuclei are a good deal shrunken and the protoplasm has lost its round vacuoles, and is changed into a clear space around the nucleus, hence the clear appearance of the clusters made up of such degenerating cells. Extreme cases of degeneration are found here and there, the nucleus having lost all its structure. On the other hand, normal or nearly normal cells are not rare; they are found in clusters containing degenerated cells; hence the appearance of the latter can not be attributed to defective preservation."

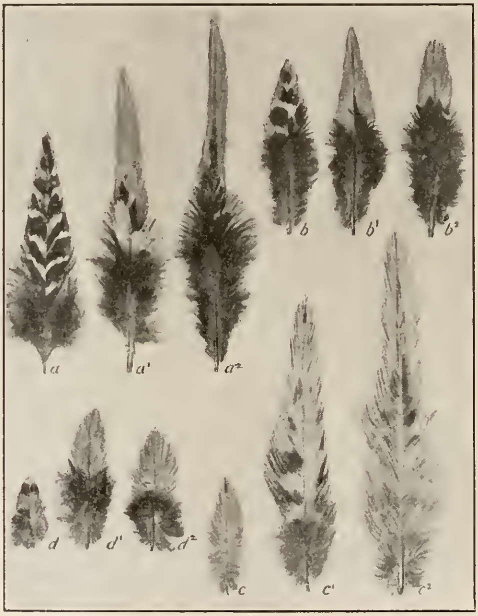


FIG. 7. Feathers of Fig. 6, *a, b, c, d,* before castration June 17, 1919; a^1–d^1, at the time of castration Sept. 13; and a^2–d^2, March 2, 1920.

After castration this bird continued, as would be expected under any circumstances, to develop cock feathering. Its condition on Nov. 12, 1919, is shown in the photograph Fig. 6, *B*. The condition of the bird on March 6, 1920, is shown in Fig. 6, *C*. It was completely cock-feathered, but its comb was two thirds full size. The condition of the feathers is shown in Fig. 7, a^2, b^2, c^2, d^2. Before the operation June 17 the feathers were intermediate in character (a, b, c, d). The back and saddle feathers were somewhat pointed and the barring broken. After the operation (Sept. 13) the feathers that came in changed completely so that the bird became strictly cock-feathered. On March 2, 1920, the change was complete (a^2, b^2, c^2, d^2). The bird was killed on March 6, 1920. Three very small pieces of testis were found on the right side; none on the left. The comb measured three and a half inches long and two inches high.

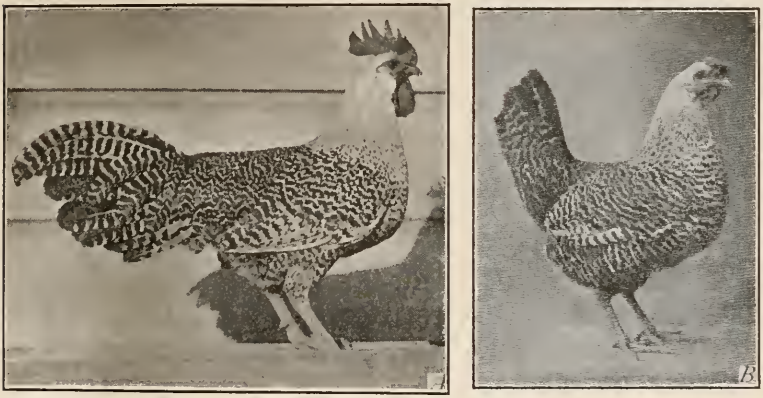


FIG. 8. *A*, normal hen-feathered male nearly one year old from the same flock as those operated on; *B*, Campine hen from Wright's "New Book of Poultry."

Dr. Nonidez has sectioned the pieces of the testes and makes the following report. "The histological study of the three small pieces found in the region of the old testis showed that they are undergoing regeneration. The condition of the seminal tubules in these pieces contrasts with that of the tubules in the testes removed at the time of castration. While in the latter only spermatogonia occur in the tubules, in the regenerating testis the normal spermatogenesis had started and all the stages, from the spermatogonia to mature spermatozoa, could be observed in the slides. The spermatozoa finally degenerate and this is probably due to the lack of an outlet in the organ. New tubules are arising near the surface of the section. The clusters of luteal cells, however, have apparently disappeared, their place being taken by the ordinary connective tissue and an enormous number of small lymphocytes which are arising in the intertubular tissue at this time. Some of these small lymphocytes become transformed into large wandering cells which usually occur in the testis of both hen- and cock-feathered birds. The disappearance of the luteal cells may be accounted for by the failure of this tissue to regenerate owing to its more or less abnormal condition at the time of castration. If some of the cells found in the sections are really luteal cells their numbers are probably too small to induce any change in the characteristics of the plumage. The relatively large size of the comb in the 'larger' bird, on the other hand, may be due, even in the case in which a very small

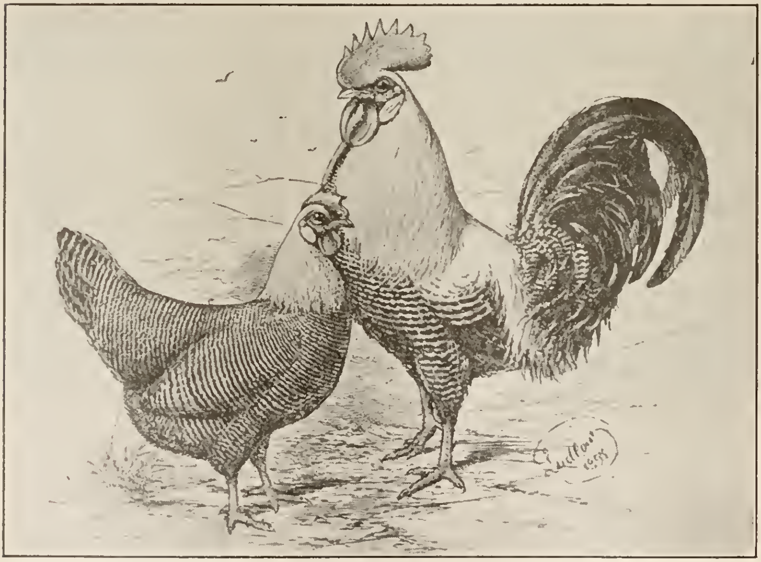


FIG. 9. Cock-feathered, normal cock and hen, from Wright's "New Book of Poultry."

piece of the testis has been left, to the influence of a secretion other than that produced by the luteal cells."

An adult cock of the stock from which my young males came is photographed in Fig. 8, *A*. For comparison I have introduced a figure of a Campine female from Wright's "New Book of Poultry" Fig. 8, *B*. Except for the neck feathers, the plumage of these two birds is barred throughout. The peculiarities of the barring in different regions can best be seen in the single feathers (Fig. 3, *a–d*).

The tail coverts of the hen-feathered cock are barred and longer than those of the hen. In this breed these feathers are not so much kept down as they are in the Sebright. The saddle feathers of the hen-feathered male are also fairly long, but broad and rounded at the end and are without hackles. The shoulder and wing bow are barred and without hackles.

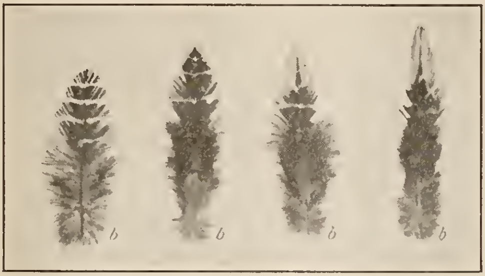


FIG. 10. Four feathers from back of a hen-feathered Campine cock of another flock. Three of the feathers show indications of cock-feathering.

The cock-feathered Campine male is shown in Fig. 9, from Wright's book. The long white neck feathers, the white back and rump feathers (both kinds pointed and with hackles), the black tail coverts, and the white shoulder are points in which the cock-feathered differs from the hen-feathered bird, and are exactly the same features that appear when the hen-feathered male is castrated.

GENERAL CONCLUSIONS.

It has been shown in the Sebright that hen-feathering is inherited. When crossed to a cock-feathered race (Game Bantams) all F_1 birds are hen-feathered, though some of them not as completely so as the Sebright. Still there is no overlapping with the cock-feathered type. The F_2 and the back-cross cocks are either hen-feathered or cock-feathered; the former showing about the same range of variability as the F_1 cocks. The failure of perfect dominance in some of the F_1 and F_2 cocks may indicate that the heterozygote has a wider range (the gene not completely dominant), or that subsidiary genes introduced from the cock-feathered birds allow a wider range of variability of the character. Only an extended series of experiments can settle this point.

In the Campines also there is evidence that the gene for hen-feathering is inherited and dominant. In a paper published several years ago (1914) by the Rev. E. L. Jones, crosses between two races of Campines are described, one of them belonging to a race (Belgium) in which hen-feathered birds are the recognized standard, the other (English) belonging to a race in which cock-feathering is the standard. The evidence seems to show that hen-feathering is dominant, also that there is segregation in the back-cross, and in F_2 as in the Sebrights. There are however serious inconsistencies in some of the results that may be accounted for on the assumption that the hens of the cock-feathered race were not pure for the character-pair in question;—an assumption that does not seem too improbable, when one recalls that the latter, the dominant type, has often been mixed with cock-feathered strains. Whether the gene for hen-feathering is the same as that in the Sebrights can, of course, only be determined by crossing the two breeds. From what I have heard it appears that in this country the Campines, even when said to be pure for hen-feathering, are not so "fixed" as are the Sebrights; for, the latter do not "break" as do the Campines unless castrated, while the Campines are reported to show quite often a few or many cock-feathers. It is recognized that when sick or in poor condition such feathers appear and may be replaced later by hen-feathers if the bird recovers. I have some feathers from an old bird of another strain, purporting to be pure for hen-feathering, that show unmistakable changes in the direction of cock-feathers (Fig. 10). At the time the bird had been confined and was in poor condition. This difference in the two races might be due to different factors, or, as seems more probable, due to the less complete dominance of the gene in the Campines (in conjunction with the rest of the hereditary complex). Possibly different modifying genes are present that fortify the action of the main gene in the Sebright. Further experiments will, I hope, settle this question.

The possible influence of other endocrine secretions must also be examined, because in mammals there is some evidence that other organs than the gonads may produce effects on the secondary sexual characters. Since the pituitary gland is one of the internal glands that, par excellence, affects growth, I have examined its condition in the three Campines and compared the organ with that in one normal hen-feathered bird of the same race. In general there is little if any difference in size between the pituitary in the normal and castrated birds; but it appears to be slightly larger in the castrates, more especially the part inclosed in the sella turcica. Since I had only one normal bird for comparison, this difference may be purely accidental; but the observation justifies further search on more ample material. Moreover, accurate measurements of the gland will be necessary before any value can be attached to such findings.

It should be recorded, perhaps, that the kidneys in the smallest and most completely castrated bird are reduced in size, but whether this bird was abnormal in this respect can not be stated.

I have not observed any change in the suprarenals in the castrated birds.

It is also interesting to note that the removal of the testes of cock-feathered birds (to produce capons) causes in them also some slight changes in the same secondary sexual characters in which they differ from hen-feathered birds. The neck and saddle feathers of the capon become longer, as do also the longer wing coverts (Goodale, Morgan). It appears, then, that even in the cock-feathered birds there is some inhibition to the growth of these feathers produced by the normal testis. This inhibition may be supposed to go so far in the hen-feathered races as to completely stop down the male plumage to that of the hen. The plumage of the hen, too, is known to be inhibited by the ovary; for, when the ovary is removed she develops the full cock-feathering characteristics of the male of her race (Goodale). Elsewhere I have discussed how these effects are brought about —whether the same kinds of secreting cells are present in the ovary and in the testes of the hen-feathered males. This question is being further studied at present and need not be considered here.

The comb and wattles are the most remarkable secondary sexual characters of the cock. Their condition is a close index of the condition of the gonad. In fact, after castration their size and the amount of blood present in them indicates how far the gonad has been removed. Even a very small piece of the testis suffices to cause them to grow to their full size. This is well shown in the pictures of two of the Campines described above, in which the comb became reduced shortly after the operation, and then slowly grew almost to its full size in the course of the following months. Its paleness showed, however, that the testes were not normal in size, and this was confirmed by autopsy. When the birds were killed a small piece of the testis was found. In the hen, too, it appears that the ovary has an inhibitory influence on the comb and wattles; for, as Goodale has shown they become larger in the ovariotomized birds. Since the testes of the normal full-grown cock-feathered bird seem deficient, or lacking in luteal cells (Boring and Morgan) it may appear that these cells do not produce the endocrine secretion that affects the comb, although if it can be shown that they do not completely disappear even in adult cock-feathered birds it may be that a much smaller amount of this secretion than is essential for suppressing the cock-feathering suffices to cause the enlargement of the comb and wattles. But if, as is said to be the case, the luteal cells disappear entirely in the adult cock-feathered bird, then there must be some other element in the testis that maintains the comb and the wattles at their maximum size. Here again is a further field for investigation.

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THE EFFECTS OF LIGATING THE TESTES OF HEN-FEATHERED COCKS.

T. H. MORGAN.

It has been shown that when hen-feathered males of certain races of poultry (Sebrights, Campines) are castrated the new feathers have the characteristics of the cock-feathered males. The operation of castration is dangerous for adult birds with large testes because the birds often die from hæmorrhage from the spermatic blood vessels, yet in order to be certain that a cockerel is going to be hen-feathered it is necessary (except when races pure for hen-feathering are used) to operate on adult birds. As these are often too valuable to risk the chances of an unsuccessful operation I have tried to find out whether the same

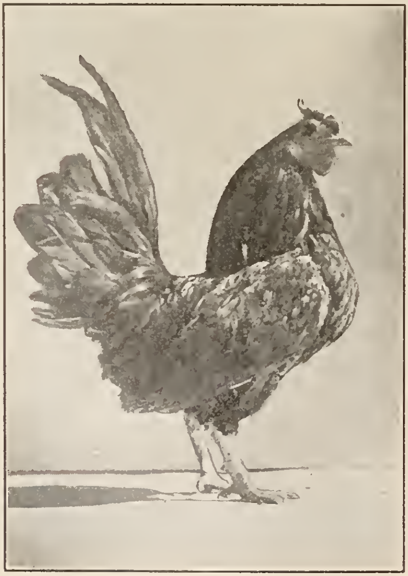

FIG. 1. Sebright whose testes had been tied off September 29, 1919; the photograph was taken April 8, 1920.

end results may not be obtained by ligating the testes. If a silk thread is tied tightly around the attachment of the testis to the body wall, thus cutting off the circulation to and from the testis, the organ degenerates and in a short time is absorbed. In order

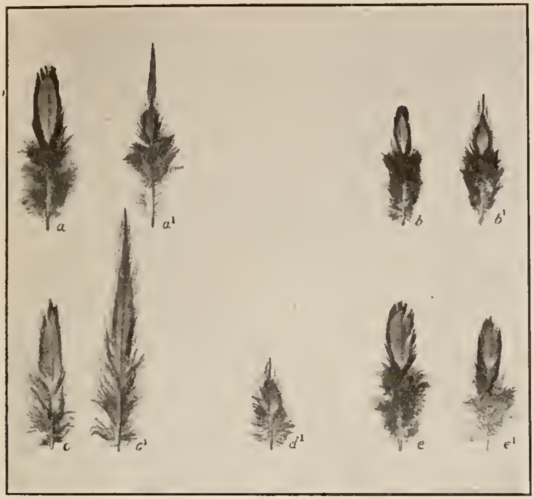

FIG. 2. Original feathers of Fig. 1, *a*, *b*, *c*, *d*; new feathers a^1–c^1.

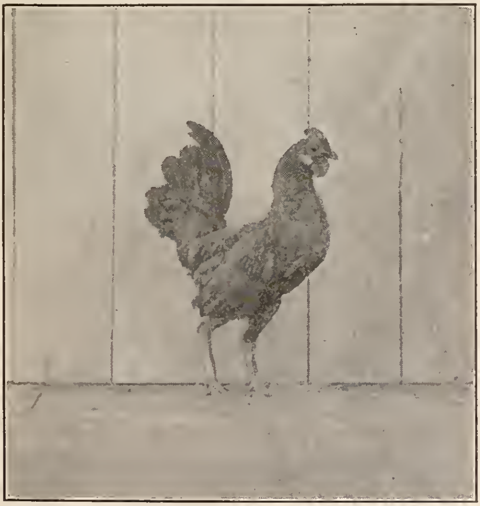

FIG. 3. Hen-feathered, back-cross, "Lamey."

that the results shall be clean cut it is necessary that the entire testis be included in the ligature, for, if even a small piece is left, it may subsequently enlarge and make the outcome less complete.

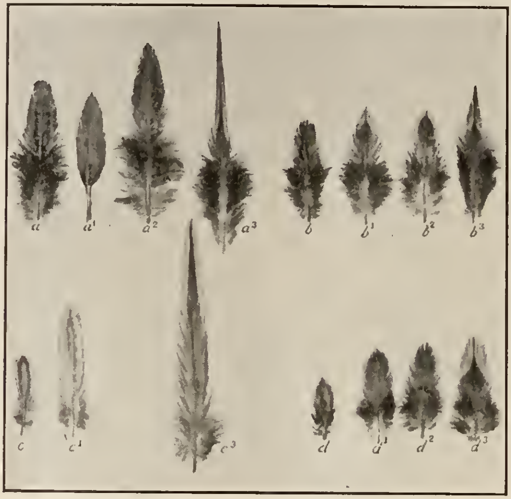

FIG. 4. Original feathers a–e of Fig. 3, and later feathers, a^1–e^1, a^2–e^2, a^3–e^3, July 7, September 17, and March 13, 1920.

In fact, as the following cases show, only one case of the four was completely successful, although all of the other three cases were sufficiently successful to cause some change in the plumage in the direction of cock-feathering.

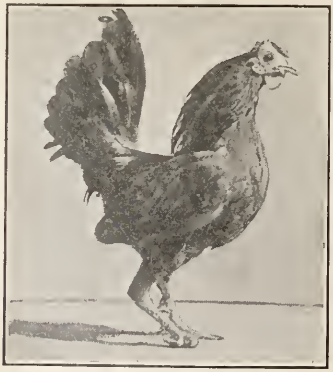

FIG. 5. Same as Fig. 3, after ligation, March 2, 1920.

The first bird was a pure Sebright male more than a year old. It was a typical bird of its race, *i.e.,* it was completely hen-feathered. This bird had been used in an experiment a month earlier to induce beri-beri, but had fully recovered. I had seen a statement in *Science* that young chicks are peculiarly susceptible to a lack of vitamines in their food and that beri-beri can be induced by a diet free from or low in vitamines. The possibility that the testes might also be affected by such a diet, and cause a change in a hen-feathered male of the same kind as that brought about by castration suggested the experiment. Two hen-feathered cocks were used. Both were fed on white rice for five weeks. No changes were observed to take place in their combs. Suddenly the effect of the diet became apparent, and in the course of

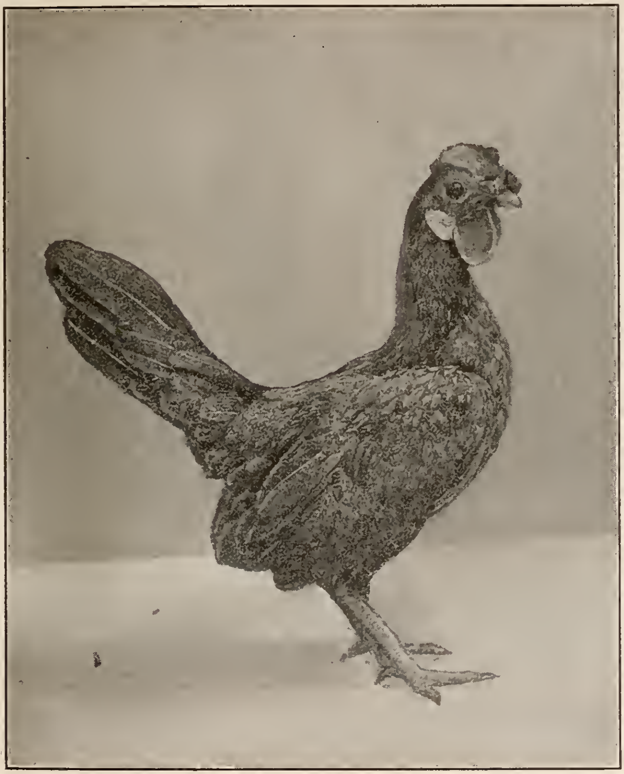

FIG. 6. Hen-feathered, back-cross, rose comb, No. 270, June, 1919.

two days the birds almost died. They lay on the floor of the cage with their muscles convulsively contracted. They were immediately fed on milk by means of a pipette and later on bread and milk. In the course of a week they had completely recovered. At the time of the operation, a few weeks later, they were active and very healthy-looking birds.

The testes of the Sebright were tied off on Sept. 29, 1919. A few feathers from typical regions were pulled out and are shown in Fig. 2, *a, b, c, c*. By Oct. 24 the new feathers had begun to come in. They were typical cock-feathers. When the last photograph was taken, Fig. 1, April 8, 1920, the bird had changed over almost completely to cock-feathering. The comb was small; not more than a third its original size. The extent to which the change had taken place is best shown in the feather-chart, Fig. 2, a^1–e^1, where one of each of the original kinds of feathers is placed side by side with the new feather from the same region.

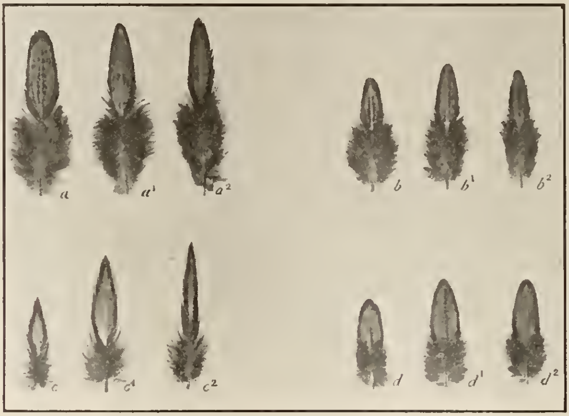

Fig. 7. Original feathers, *a–d* of Fig. 6; and later feathers, a^1–d^1, November 15, and a^1–d^2, March 15, 1920.

The second bird operated upon, called "Lamey," Fig. 3, was a yellow, back-cross, hen-feathered cockerel, one year old. Its testes were ligated in May, 1919. It went over towards cock-feathering during the summer (July, 7), as shown by the feather-chart, Fig. 4, a^1, b^1, c^1, d^1. Both the old and the new feathers were present at this time and one of each from typical regions is shown beside the other. As the change had not gone as far as expected, had the ligation been entirely successful,[1] the bird was opened on September 17. Its feathers at this time are shown in Fig. 4, a^2, b^2, c^2. Pieces of the testes were found present and an attempt to remove them was made. During the winter the bird showed somewhat further changes in his plumage as new feathers developed. On March 13, 1920, he appeared as shown in Fig. 5. The condition of his feathers is shown in Fig. 4, a^3, b^3, c^3, d^3. These show that the change had gone much further, as the smaller size of the comb would lead one to expect. He was killed on March 17, and examined. On the left side there was a small piece of testis about one fourth inch in diameter. On the right side there was a chain of very small pieces of testis. The comb measured two inches in length and one fourth inch in height. The wattles measured three quarters of an inch in diameter.

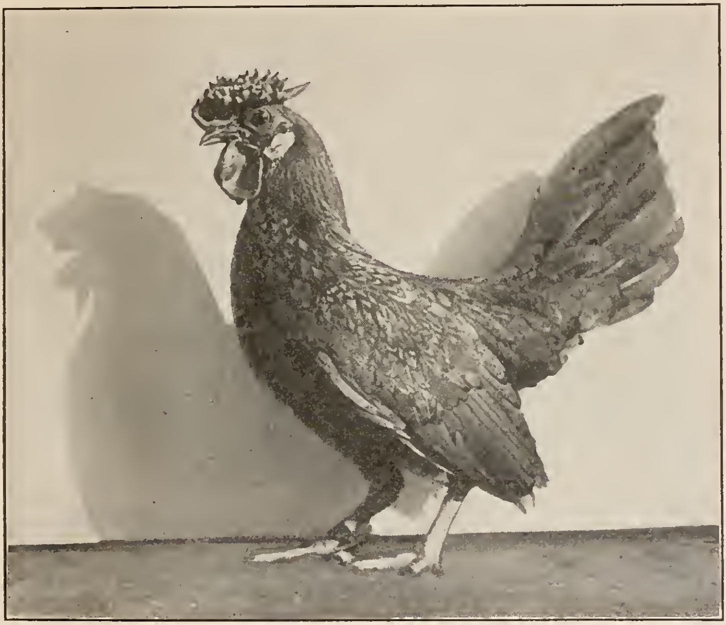

FIG. 8. Same bird as Fig. 6, November 15.

The third bird, (No. 270), was a rose comb, back-cross, hen-feathered cockerel, a year old (Fig. 6). The bird looked somewhat like a Sebright, but the yellow centers of the feathers were much mottled making the general effect of the plumage darker than that of the Sebright. Ligatures were put around the testes on July 10, 1919, but unfortunately one of them did not completely encircle the end of one testis, so that a piece was constricted off. This piece was left in position at the time. The feathers that came in (Sept.) underwent very little change, the centers becoming somewhat clearer, Fig. 7, *a*^{1}–*d*^{1}. The presence of a large comb made it certain that a considerable amount of the testes had remained active. On opening the bird (Nov. 15), no pieces of the left testis were found. On the right side there was a testis highly vascularized, attached to the body wall. It was removed and after the operation the comb became smaller; but after a while began to grow, until by March 8, it was quite large again. The bird might still be said to be almost completely hen-feathered (Fig. 8). It was killed and opened on March 17, 1920. On the left side was found a large, detached piece, berry-like in appearance, undoubtedly a regenerated testis. On the right side

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[1] The condition of the comb showed that some testicular tissue remained.

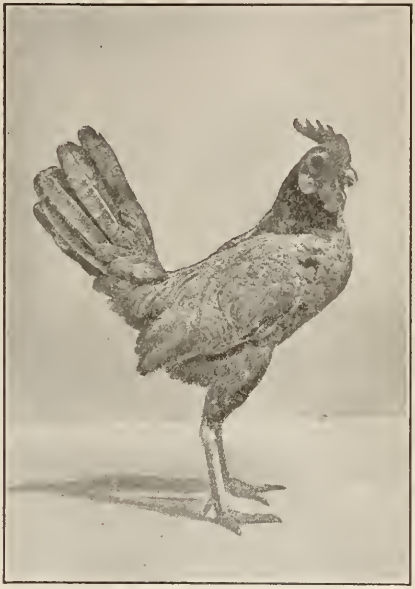

FIG. 9. Hen-feathered, back-cross, single comb, No. 62.

a testis appeared almost normal in size. The comb measured one and a half inches high, and two and three quarters inches long. It is evident that the failure to change over except to a very small extent was due to the failure of the operation.

The fourth bird, No. 62, was a yellow, back-cross, single comb, hen-feathered cock, Fig. 9. Its testes were ligated on July 10, 1919. He showed at first some effect of the operation, but his comb soon began to enlarge again. On November 15, he was still hen-feathered (Fig. 10). A comparison of the feathers of July 10 with those of November 15, Fig. 11, *a*, a^1, *b*, b^1, etc., shows little change. When opened at this date no trace of the testis was found on the left side. On the right side a large piece of testis-like tissue was present in a sheath tightly adherent to the body wall. It was opened and some very loose tissue(?) taken out. This was preserved for examination and the bird was closed up. It was kept alive until March 15. Its feathers at this time are shown in Fig. 11, a^2, b^2, c^2, d^2. The comb was large and the bird appeared to be nearly hen-feathered. It was killed and examined. On the left side there were two small pieces of testis which together were no larger than a pea. On

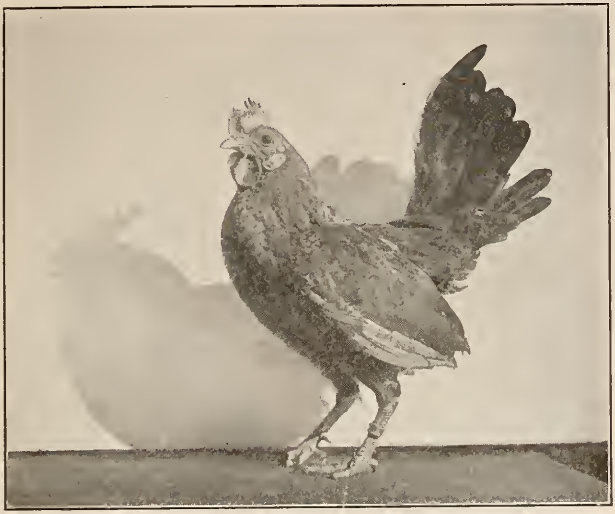

FIG. 10. Same as Fig. 9, November 15.

the right side there was an almost complete organ. The comb was large, measuring half an inch in height and two inches in length. Each wattle was one inch across. The condition of the testes accounts for the failure of the bird to change over.

These few experiments suffice at least to show that it is possible to cause the total degeneration of the testis if ligation is successful; and further that unless the testes are entirely removed the complete change-over to cock-feathering does not take place.

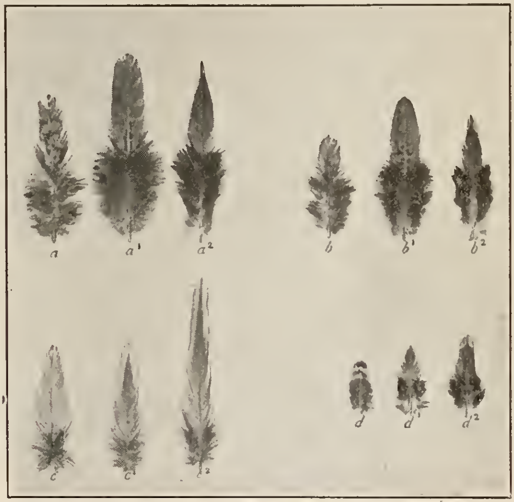

FIG. 11. Original feathers, *a–d*, July 10, and later feathers November 15, a^1–d^1, and March 15, a^2–d^2.

They also show, however, that some change in the direction of cock-feathering will take place if some of the testis is removed, and the amount of the change is roughly proportional to the amount of testis present. The comb is a sure index of the amount of the testes left. It appears that a smaller amount of the testis suffices to keep the comb at or near its normal size than to keep the feathers true to type for hen-feathering. For a complete change to cock-feathering it is essential that all of the testis be removed. These results accord fully with the results that I have obtained by castration. The chief significance of the results is to show that adult birds can be successfully used if ligating is employed instead of castration.

THE GENETIC FACTOR FOR HEN-FEATHERING IN THE SEBRIGHT BANTAM.

T. H. MORGAN.

Crosses between Sebrights and Game Bantams had shown that the dominant gene for hen-feathering is inherited as an alternative to cock-feathering, but the number of F_2 and back-cross cocks that were obtained was too few to settle the question whether one or two pairs of genes for hen-feathering were involved. The situation has been recently discussed in my paper on "The Genetic and Operative Evidence Relating to Secondary Sexual Characters," Carnegie Publication, No. 285, 1919. I am now able to add a small amount of new data to that previously recorded, although I regret that even the combined data still do not show clearly whether one or two factors for hen-feathering are involved.

During the summer of 1919, I back-crossed four hens (out of Game by Sebright) to a pure Game Bantam cock bought from the same breeder from whom the other Games had come. The chicks were hatched in June and July, 1919, and the following records were made on March 22, 1920, when the new birds had fully attained their adult plumage.

Five of the cockerels were hen-feathered, and three were cock-feathered. There was no difficulty in separating the cockerels into two classes. Only one bird fell within the group of hen-feathered cocks that in my previous paper I sometimes spoke of as "intermediate." This means only that some of the back and saddle feathers were more pointed than are those of pure Sebright cocks. Their ends may show on each side of the tip a narrow edging lacking barbules. Previously the same back-cross gave two hen-feathered and seven cock-feathered males. The two records together give, therefore, 7 hen-feathered and 10 cock-feathered males.

For a single factor-difference the expectation for 16 birds would be 8 hen-feathered and 8 cock-feathered birds. The numbers obtained are in fair agreement with this expectation. If two factors are necessary for hen-feathering, the back-cross expectation would be 4 hen-feathered to 12 cock-feathered birds, which differs so much from the results obtained that the assumption of a single factor-pair seems the more probable.

In former years I have obtained 127 F_2 offspring from the above cross of Sebright by Game Bantam and its reciprocal. These have been recorded in the Carnegie paper (Table I., page 19). There were 55 males in the records. If a single pair of not-sex-linked genes was involved in the cross, the expectation in F_2 would be, three cock-feathered males to one hen-feathered one. If two pairs of genes were involved either one of which is sufficient for the development of hen-feathering the expectation in F_2 would be 9 to 7; if both are necessary the expectation is 15 to 1. There were 29 hen-feathered, and 26 cock-feathered birds. It was pointed out that these data seem in better agreement with a two-factor pair, than with a one-factor pair assumption. But, as pointed out above, the old and the new data combined for the back-cross, appear to fit better the single factor pair assumption.

There is one other possibility that may be considered. If there is one dominant factor, D^1, for hen-feathering in the Sebright, and a modifier that intensifies the action of D^1, but alone or in duplex does not produce hen-feathering, the expectation for F_2 is 12 to 4, and for the back-cross 1 to 1. The former ratio, which is of course the 3 to 1, does not fit the case any better than one factor alone, although it might help to explain the intermediate type of male, as due to the presence of a normal allelomorph of the intensifier.

Color Inheritance in the Cross of Sebright to Game.

It is now possible to add a few more cases to the data for color-inheritance. Four back-cross hens were reared (1919), that fall in the former classes (Carnegie Publication, No. 285, pages 19–20) *B, G, A, A*. One of the four is almost exactly like the game-hen. The cockerels fall into the classes, *B, G* (or *H*), *S, S, S, K, E*.

Of these 11 back-cross birds two were almost exactly like the game (one female, one male). For two pairs of color factors, the game type is expected in the back-cross in one fourth of the classes, *i.e.*, in 3 out of 12, which agrees fairly well with the results. For three factors for color, the game type is expected in the back-cross once in eight times. The numbers are too small to be decisive for either case, but as pointed out before, the main color difference between the two races may not be more than 3 or less than 2 factor-pairs.

In the earlier experiments one game appeared in F_2 out of 49 females, which is somewhat favorable to the assumption of three factor-pairs. The new data are not sufficient in amount to settle the question between two or three factors, but the latter is perhaps somewhat more probable.

Proof that the Character for Hen-Feathering is not Sex-Linked.

The proof that the factor (or factors) for hen-feathering involved in this cross is not carried by the sex-chromosome can not be deduced from the evidence of the F_1 males in the cross and its reciprocal, for, both the F_1 males would get a Z chromosome from each parent, and therefore would be alike in both cases. But in the F_2 generation the proof is given, for when the cross is made one way, only hen-feathered males are expected, and when made the other way (reciprocal) one hen-feathered to one cock-feathered male is expected, if the gene concerned is sex-linked. The F_2 results in both crosses show, however, both hen-feathered and cock-feathered males; hence the factor (or factors) involved is shown not to be carried in the sex-chromosomes.

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BIOLOGICAL BULLETIN

NOTES ON PEDICULUS VESTIMENTI.

KATHARINE FOOT.

The following notes are a curtailed record of two years' work on the body louse, when I was serving as a volunteer in the American Red Cross in Paris.

I undertook this work at the request of Dr. Alexander Lambert who was then at the head of the research department of the American Red Cross.

My reports were presented as contributions from the Foot and Strobell laboratory because a legacy left me by my friend Miss E. C. Strobell made it possible for me to give my entire time to the work and to pay the current expenses of my laboratory.

I am greatly indebted to the late Professor Blanchard for giving me space in his laboratory at the École de Médecine and for many other courtesies in connection with my work, and I am much indebted also to Professor Langeron, a member of Professor Blanchard's staff in the department of parasitology.

Although any official obligation to the Red Cross was ended when the research department was closed it has in no way altered my sense of obligation to continue my work with the hope of contributing some data that may serve in adding at least some control to the activities of the body louse, for this insect is nothing less than a curse not only to the armies but to those races which are forced to live under unsanitary conditions.

The first few months of investigation were necessarily devoted to a study of the life cycle of *Pediculus vestimenti,* an accurate and detailed account of which had already been published by several English investigators. Many years of experience in raising other species of Hemiptera led me to adopt slightly different methods of laboratory technique than those used by other investigators in their breeding experiments. These methods are described in a recent note on the "Spermatogenesis of *P. vestimenti*" (Foot, '19).

It is impossible to breed lice without daily feeding them on human blood, and in the above mentioned note are stated the difficulties I encountered in finding a host. I finally secured the services of an old French sailor who came to my laboratory every day to allow them to bite his arm for one hour.

An amusing and perhaps significant behavior of the lice during the feeding hour was emphasized in my report to the research department of the Red Cross because it suggested a type of experiments that might be of some scientific value and needed the coöperation of a medical adviser. The old sailor (my food supply) was addicted to some drug—whether alcohol, absinthe, morphine or what not—it was impossible to determine, but the effect was unmistakable both on the sailor and the lice. The sailor slept the entire hour and the lice fought one another with apparent vicious intent. They showed no indication of seeking one another, but if two met while wandering restlessly about each would seize the other by the head and thorax, and claw with vicious activity, at the same time clinging with such tenacity that it was often impossible to separate them without injury to one or both. The combatants were either two males, two females, a male and a female or an adult and a nymph.

I learned later that this belligerent aspect of lice was appreciated by some of our soldiers and that a "cootie fight" in the trenches was one of the few recreations possible and afforded not a little amusement. Two sailors contributed each a cootie and they were placed on a small hand mirror, the ensuing combat been watched with keen interest and I suspect that the sense of proprietorship extended to the point of each owner betting on his own cootie. The boys thought the fighting spirit of the lice due to the fact that the two had been raised on different hosts, but I have tested this suggestion with negative results and I am inclined to think it merely an expression of some discomfort, due in the one case to the change from skin to glass and in the case of the old sailor it seemed to be a direct response to some abnormal condition of the blood. In this case it seriously interferred with my breeding experiments, for the lice not only injured or killed one another but it prevented their feeding normally. When fed only one hour during the twenty-four, they (as a rule) feed the entire hour or stop only for short intervals—they pass each other and walk over each other without showing any mutual antagonism. The process was quite reversed in the case of the old sailor, for frequently, after feeding only five minutes, the fighting commenced and continued the entire hour. This phenomenon afforded not a little amusement in the laboratory and was unhesitatingly attributed to the sailor's blood, for to avoid the possibility of its being due to any foreign substance on the skin, the arm was always carefully cleansed over the area used for feeding.

In order to secure peaceful feeding it was necessary to place a single louse in each of the glass rings in which they were confined while being fed.[1] The above described curious effect of the abnormal blood of the host was not limited to the feeding hour, for the lice frequently attacked one another in the cages in which they were confined. Further, I found it exceedingly difficult to raise the young lice—they sometimes refused to bite at all and often died two or three days after feeding.

Being finally convinced that the blood of the old sailor was a most unwholesome and even dangerous food supply and that my work was being seriously hampered by conditions I could not control, he was dismissed and exchanged for a mild old refugee from Chateau Thierry, who neither smoked nor drank. From that day I encountered no such difficulties in raising the lice. I could feed ten or more in a single ring and they fed together and lived together most amicably—the adults no longer attacked the nymphs nor did they attack one another.

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[1] In the above mentioned note on the spermatogenesis these rings and the cages are described as follows: "When feeding the lice I at first used the usual method of putting a number in a tube, inverting the tube on the arm and holding it securely in place to prevent the lice from escaping. I found this method unsatisfactory for several reasons and devised therefore quite a different technique: Lice cannot crawl up a glass surface if it is clean and are therefore perfectly safe in a glass ring even if it is only 2 cm. high. I had such rings made to order and fastened them securely onto the arm with melted paraffine (photo). In this manner several different experiments can be conducted at the same time and the generations can be kept separate—further the lice can be conveniently studied with a lens during the hour they are feeding. For the remaining twenty-four hours they were kept in a Pasteur incubator at a temperature between 27° and 29° C. While in the incubator the lice were kept in cages such as those used in the laboratory for raising various insects. This cage is the tube de Borel, in which is placed an inner tube for the insects, this being held in the center by absorbent cotton which is kept wet to insure sufficient moisture. I found the use of absorbent cotton very inconvenient and replaced it with a short tube having an aperture at both ends with a lip at each end sufficiently wide to center it in the tube de Borel. The inner tube in which the insects are kept is dropped into this shorter tube and an inch of water kept in the tube de Borel."

After describing this phenomenon in my first report I concluded "These observations indicating that anything taken internally that may affect the character of the blood may also act directly on the louse suggest a line of experiments that might be worth trying and that I should like very much to undertake if I may be allowed the necessary guidance of a physician. The lice are so exceedingly fastidious in their diet, I would like very much to try the effect of certain drugs given to the host. If some simple drug can be found that when taken by a soldier will kill his lice or prevent their propagating it may be a helpful factor in combating the pest."[1]

A striking evidence of the sensitiveness of the lice to their food is the fact that although they will suck the blood of many animals they are not adequately nourished. Many investigators have attempted to feed them on animals and the results from these experiments are contradictory, authors differing as to whether the lice will or will not bite a definite animal. In the cases in which they have been induced to bite no evidence is given as to how long they survive.

In his classic work on *Pediculus* Nuttall ('17) has summed the evidence from experiments on the monkey, dog, rabbit, guinea pig, rat, mouse, fowl, pigeon and swallow—page 113.

My own experience has been that the lice will suck the blood of every animal I have tried but were not properly nourished, for they lived only a few days longer than under complete starvation; further the blood of the guinea pig as stated by other investigators is not only malnutritious but toxic for, as a rule, the lice die within 24 hours after feeding.

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[1] I have recently learned that a French doctor conceived the idea of attacking the lice by this method but was unable to spare time for the experiments.

At intervals during a period of two years I have fed *P. vestimenti* on rabbits, guinea pigs, rats, mice, fowl, pigeons and canaries. In addition to feeding them on the blood of these animals I have tried to give them human blood by inserting a small piece of absorbent cotton under the most superficial layer of the epithelium of a young white mouse and dropping a few drops of fresh human blood upon the cotton. They bite the white mouse readily and get the human blood, as was shown by initial experiments with physiological salt solution stained with eosine—the colored solution in the alimentary tract demonstrating the possibility of their getting human blood by this method. A fresh mouse could be used each day, avoiding the possible criticism of the mouse itself becoming infected. Illness prevented my carrying these experiments to a satisfactory conclusion and I therefore do not know whether life can be much prolonged by this method. It however justifies further experimenting, for it should be of value in connection with those problems where it is imperative to keep the lice alive for definite periods after feeding them with infected blood. Further it should be of value in determining whether the lice themselves are infected, or whether the infection is confined to the blood in the alimentary tract, the latter acting merely as a mechanical transmitter. Many authors believe that this is the sole method of infection—scratching the bite and thus inoculating the victim with the feces of the louse being the sole danger—the bite alone being harmless. Nuttall ('17, page 61) quotes one experiment where a man was bitten by fifteen thouand lice which had fed on a relapsing fever patient and a second case in which a thousand lice were used. In neither of these cases was the host infected.

It is an established fact that the louse is the carrier of at least three diseases. It has been demonstrated for typhus fever by Nicolle ('09), Nicolle, Conte and Conseil ('10) and others. It has been demonstrated for recurrent fever by Sergent and Foley ('10), Sergent, Gillot and Foley ('11), Nicolle, Blaizot and Conseil ('12) and others.

It has been demonstrated for trench fever in the Report of the Medical Research Committee of the American Red Cross.

In addition to the above mentioned established cases lice have been suspected of carrying nearly a score of other diseases. In view of the fact that the louse is not only a dangerous pest but that its bite causes subsequent effects that may be torture to the victim, the most imperative need is to persist in the search of a means of eliminating or at least limiting them, in spite of the many discouraging experiments which give only negative and disappointing results. Even a negative result may be of value to future investigators as one false step to be avoided and it is in this spirit that I have attacked the problem.

Innumerable experiments have been tried to make the host distasteful to the louse by various drugs applied to the skin or clothing but these have been so far from satisfactory that the most practical method at the front and elsewhere has been the bath and a complete change of clothing. This gives at least temporary relief, but to the poor victim who is sent back to the trenches it is of short duration. I have talked to many soldiers who have had sad experiences with *Pediculus,* and all appreciated the delousing methods in use but expressed the need of some method by which each soldier might individually control the pest even when compelled to live in their midst. I have often been impressed by the keen appreciation of our soldiers towards any effort to kill the "cursed louse." One amusing experience is worth recording. I had talked for nearly half an hour with two poor fellows who had just come from an area of the front which had no delousing outfit. They did not complain but merely stated how impossible it was to get an entire change of clothing and that a clean shirt one day and some other garment a few days later was a useless effort at delousing. They both had suffered greatly and were depressed at the thought of returning to the front the next day. They appealed to me for help and asked what experiments I was trying. When I told them I was experimenting with the hope of finding some drug that when taken by the soldier might make him distasteful to the cootie, one of the soldiers jumped to his feet exclaiming "My God! that is a good idea! what shall we take?"

So many investigators have experimented with the external use of drugs that it makes further effort seem almost hopeless and gives very little encouragement that the experiment of internal use of drugs may be of practical value though it may have scientific interest.

As stated above these experiments were suggested by the apparent direct response of the louse to the blood of the old sailor who was habitually under the influence of some drug. Further the toxic effect of the guinea pigs' blood and the non-nutrition of the blood of other animals led me to suspect it might be possible to find a drug that given to the host would make his blood distasteful or malnutritious to the lice, causing them to leave their victim, or it might be sufficiently injurious to the reproductive organs to inhibit multiplication. If the sperm or eggs can be injuriously affected this might prevent fertilization and so control the most effective phase of infestation. The danger from this cause is obvious from the fact that a single female lays about ten eggs a day[1] and as soon as these hatch (in five or more days) the nymphs begin to bite at once—feed during the day and night, and when mature (after about 20 days) the new life cycle begins. Thus the danger from a single fertilized female is apparent.

The mature insects, which are about 3 mm. long, can be easily found and as they are by no means active they can be readily caught and killed. The eggs and nymphs, however, cannot be so easily eliminated, for they are so tiny they may escape detection. It is obvious therefore that any offort made to inhibit or even limit reproduction is justified and worthy of patient experiment.

The first difficulty encountered was to find a host who would be willing not only to feed the lice but to consent to being drugged daily for at least a month as that was the period selected in order to determine any possible effect not only on the rate of reproduction but on the life of the nymphs as they may possibly be more susceptible to the food supply than are the adults. After finding a suitable host the experiments were conducted in the laboratory on a limited number of pairs of *P. vestimenti* and in order to detect any deviation from the normal a daily record was kept of the behavior of the lice being thus fed on presumably medicated blood.

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[1] Nuttall, '17, page 130—"We may therefore conclude that under optimum natural conditions 275 or 300 eggs represent the normal number of eggs which a female is capable of laying and that during the greater part of her oviposition period she days 9 to 12 eggs a day or an average of 9.7."

By this method three drugs were tested, Quinine, potassium iodid and sodium salicylate.[1] Of these three drugs quinine (which was suggested by Dr. Alexander Lambert) caused apparently the most abnormal response. As stated in my report "biting during the hour was less frequent and for much shorter periods. The nymphs were very restless, making frequent efforts to escape under the edge of the glass ring in which they were confined." I concluded my report as follows: "Although the lice do not bite normally and do not reproduce normally the toxic effect of the quinine is not sufficiently injurious to eliminate them. The most promising result is the very evident effort of the young nymphs to escape and in view of the well-known and persistent assertion that lice avoid some healthy people and leave hosts who have contracted certain diseases, it is possible that this effort to escape may indicate a distaste of the blood which may cause lice to leave a host who is taking quinine."

Potassium Iodid.—The experiment with this drug (appendix, p. 273) was less promising than the quinine experiment, the lice showing no desire to escape—they did however attack one another during the feeding hour, this presumably being an expression of some discomfort. The results from this experiment as stated in my report are as follows: "The only abnormal feature in the life history of two generations of lice was the marked tendency of the first generation to attack one another during the feeding hour. There is no evidence that potassium iodid taken by the host is either injurious or distasteful to the lice."

Sodium Salicylate.—The experiment with sodium salicylate (appendix, p. 274) was of interest merely because the sexes of the generation which was raised from four pairs of lice was most unequal, showing a very small proportion of males. These results would be of interest if the sexes are normally equal but this is an open question in view of Hindle's conclusions as to their normal variable inequality. Nuttall ('17) in giving a summary of the data as to the sexes says " the proportion of the sexes as determined by raising experiments have yielded contradictory results," and this he thinks is due to the small broods of the experiments. This criticism justly applies to Hindle's results, whose conclusions are not convincing because they are drawn from broods which in no case reached 50 per cent. of a normal generation. The number of individuals in his 25 broods quoted by Nuttall are as follows: 2, 4, 6, 9, 9, 10, 11, 12, 15, 17, 24, 25, 26, 27, 29, 30, 31, 32, 34, 36, 38, 45, 48, 64.

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[1] See appendix, page 272, for brief records of these three experiments.

Nuttall ('17) found the sexes nearly equal, though his broods also were small. My own results are in harmony with Nuttall's though I am able to give only one brood in evidence. In this one experiment, however, I attempted to determine the sex of every individual that was hatched and succeeding with 92 per cent. of a brood of 125 lice. This was made possible by using a slightly different method from that of other investigators. As stated in my report (Foot, '19), "Instead of waiting for the nymphs to mature in order to determine the sex they were dissected at any stage that was convenient and those that died were not discarded but dissected at once and their sex recorded." One hundred and twenty-five eggs were hatched and the sex determined for one hundred and fifteen (62 males and 53 females). Should repeated experiments with sodium salicylate show that the females *invariably* predominate we might then conclude that the drug is in fact more injurious to the males, for Hindle's broods were either all males, all females or males and females. If therefore a drug *constantly* effects one sex it must be injurious whether the sexes are normally equal per Nuttall or variably unequal per Hindle.

The most practical method of testing a drug and of testing the possibility of selection by the lice is to find a family where all the members are infested and to select one member for experiment. This is not as difficult as one would suppose, for there is a popular belief among the most ignorant class that lice are conducive to health—that their relation to disease is not only preventive but curative. These people, therefore, welcome them as friendly guests—a degree of hospitality it is difficult to understand. That this belief actually exists is proved by the fact that a generous bribe is needed to induce the host to face the possibility of any treatment causing his lice to decamp.

It is self-evident that this situation affords an ideal opportunity for experiment and I have succeeded in securing the services of a young French doctor who has just commenced to practice and lives in a suburb of Paris where there is a large working class and where the popular belief as to the advantages of lice is in evidence. We are thus equipped to experiment not only with medicated blood but with the external use of drugs, which method of attack, however, has been so thoroughly tried and proved so disappointing that there is very little inspiration for further effort. I have repeated many of the experiments in the laboratory by applying the drugs on the area of the skin used in feeding and these efforts have only confirmed the negative results of other investigators.

I also experimented placing relatively impervious substances on the arm of the host to determine whether the lice would be aware of the human skin below and bite through the substance. One experiment was interesting as indicating that the lice realized the human odor. A piece of the skin of an apple was used and it was scraped until the layer was so thin it was almost transparent. After this was put on the arm of the host the lice were placed on it and showed no indication of being conscious of the proximity of the human skin—they not only showed no effort to bite but were indifferent and inert. After 30 minutes the apple skin was reversed and the surface which had been next to the human skin was placed uppermost. When the lice were placed upon this surface their behavior was entirely different—they were very active, running excitedly around in the confined area and this showing an indication of a sense of the human odor which undoubtedly adhered to the surface of the apple skin which had been for 30 minutes in contact with the arm of the host. The lice however showed no effort to bite through this foreign substance, though the experiment seemed to indicate that they are susceptible to an odor and is evidence in favor of the assertion that they avoid some people and that this selection may have some connection with a sense of smell.

I was fortunate in securing an opportunity to experiment with a case of this so-called immunity from attack by lice.[1] If we could find a genuine case of immunity, it is self-evident that a discovery of its cause might be of a great practical value.

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[1] See appendix, page 274.

The case I was able to study was that of an intelligent American soldier (Sergeant Du R.) from one of our Western States. He was thoroughly convinced that lice avoided him while attacking his companions with whom he was in close contact. As stated in my report he had been in France two years, was many months at the front and frequently exposed to the pest. He slept with his brother for about 3 months, during which period his brother was infested with *P. vestimenti* and suffered greatly. Later he slept for two weeks between two soldiers who were infested and the lice were also in the blankets. At no time was he bitten, nor did he find lice on his person, though he frequently searched for them.

In investigating his case my first disappointment was to find that the lice by no means refused to bite him, but apparently found him as tempting a host as any of the nine I had used in my breeding experiments. The next step was to attempt to find some explanation of his assumed immunity from attack. The first surmise was that he might be classed with those cases in which the bite is not followed by itching, and therefore he lacked the unmistakable evidence of their presence and was deceived as to his immunity. That explanation was quickly proved erroneous, for the itching from the bites was normal and very distressing.

A second search for an explanation was to test whether the lice could bite on all parts of Du R.'s arm, for if even a portion of his skin were abnormally thick it might give him at least some protection from attack. In my experience lice cannot bite through skin that is a bit callous, such as the distal end of the thumb and certain parts of the palm of the hand. Both adults and nymphs were fed on various areas of Du R.'s arm from the wrist to the shoulder and the biting was entirely normal in each case.

The two above mentioned suppositions could be easily tested but it was not possible to give a conclusive answer as to whether Du R.'s blood was sufficiently injurious to the lice to be offered in evidence of his claim that he was immune from attack and he cannot therefore be listed as an actual immune case. As stated in my report "the death rate for the ten days feeding is certainly abnormally high. Of the 36 used for the experiment 26 died and as most of these died before the second moult and several died while moulting, the facts seem to indicate that Du R.'s blood does not nourish the lice normally but it does not warrant assuming that it adequately explains his apparent immunity." Unfortunately his case could not be further investigated as his duties called him from Paris.

I am now having a search made among the French families that are infested, hoping to find one in which a member is immune, for such a case would be a rare chance for experiment.

It is humiliating to admit that the above report of a two years' fight with *P. vestimenti* seems to leave the louse the victor, but this war is not over nor is there the impediment of an armistice for many earnest investigators are still fighting the pest and this encourages further effort.

Appendix.

The following reports were written for the American Red Cross while I was connected with the research department; as further experiments with drugs and also with so-called immunity are still in process the details of these reports may be of value for comparison with further results.

On the Reaction of Lice to Quinine in the Blood of the Host.

Host: Julienne F., age 30 years.

Quinine: Quinine sulphate. Prescription by Col. R. P. Strong. 15 grains daily.

No. of Lice: 3 ♂, 3 ♀, collected from a healthy woman at St. Louis Hospital, January 30, 1919.

Feeding: One hour daily. The above three pairs were kept from February 1 to February 12 and during that period were seen to mate six times. No. of eggs deposited, 69. No. of eggs hatched, 38 (55 per cent.). (Normally more than 75 per cent. of this generation are hatched.) Ten of the above generation fed one hour daily from February 13 to March 5 (21 days). Biting during the hour was less frequent than normal and for much shorter periods. The nymphs were very restless, making frequent efforts to escape under the edge of the glass ring in which they were confined. (Normally they feed quietly and make no effort to escape.) After the first moult they fed much more normally. 2 died before the first moult. 1 died before the second moult. 3 died just after the second moult.

The remaining four had the third moult between March 1–4 (3 ♀, 1 ♂).

Eggs Deposited:

March 4th ……	1	
March 5th ……	3	
March 6th ……	6	
	10	(Illness of the host closed the experiment.)

Hatched: 3.

Results: Although the lice do not bite normally and do not reproduce normally the toxic effect of the quinine is not sufficiently injurious to eliminate them.

The most promising result is the very evident effort of the young nymphs to escape and in view of the well-known persistent assertion that lice avoid some healthy people and leave hosts who have contracted certain diseases, it is possible that this effort to escape may indicate a distaste of the blood which may cause lice to leave a host who is taking quinine.

The Reaction of Lice to the Blood of the Host who is Taking Potassium Iodid.

Host: Helen P., age 25 years.

Potassium Iodid: Saturated solution in water. Five drops three times daily. Prescription by Col. R. P. Strong.

No. of Lice: Four males and four females. Collected from healthy woman at St. Louis Hospital, April 2, 1919. The lice were fed one hour daily. Their behavior during the feeding hour was not normal. After feeding 10 or 15 minutes they fought at intervals during the entire hour, though they never apparently injured each other. The combatants were either two males, two females, or a male and a female. They seize each other by the head and thorax and it is frequently very difficult to separate them. After seven days feeding on the presumably medicated blood, the eggs were collected daily until 104 were deposited. The parent lice were then discarded. Seventy-five per cent. of the eggs hatched and forty of the nymphs were reserved to raise to maturity. These fed normally and showed no tendency whatever to attack each other, though twenty were fed in one small tube. A normal number reached the mature stage and the experiment was then closed.

Results: The only abnormal feature in the life history of two generations of lice was the marked tendency of the first generation to attack each other during the feeding hour. There is no evidence that potassium iodid taken by the host is either injurious or distasteful to the lice.

The Reaction of Lice to the Blood of the Host Taking Sodium Salicylate.

Host: Helen P., age 25 years.

Sodium Salicylate: Prescription by Col. R. P. Strong, 15 grains daily (5 grains 3 times a day).

No. of Lice: Four males and four females. Collected from a healthy woman at St. Louis Hospital, May 14, 1919. The lice were fed one hour daily. After seven days feeding on the presumably medicated blood, the eggs were collected daily until 159 were deposited. The parent lice were then discarded. Eighty-five per cent. of the eggs hatched and twenty of the nymphs were reserved to raise to maturity. Sixteen of these survived and after they reached the mature stage the experiment was closed.

Results: Only one possibly abnormal feature has been observed and the experiment must be repeated to determine whether it is due to the sodium salicylate. More than seventy of the F_1 generation were dissected for cytological study and a surprisingly small per cent. were males. After dissecting about thirty the following exact record was kept of the last thirty-nine dissected. Of these thirty-nine two only were males.

A Case of So-called Immunity to Attack from Lice.

There seems to be trustworthy evidence that many people are immune to attack from one or more species of insects that feed on human blood.

Nuttall ('17) gives the following data in relation to *Pediculus*

Record Showing the Proportion of Males to Females.

Date.	Stock.	Age.	Males.	Females.
6/15/19	29	7 days		5
6/18/19	"	" "		4
do.	"	Just after third moult		1
6/19/19	"	" " " "		3
do.	"	7 days		6
6/20/19	"	Just after third moult		6
6/21/19	"	" " " "		2
do.	"	8 days	1	
do.	"	7 days		3
6/22/19	"	Just after third moult	1	1
do.	"	9 days		3
6/23/19	"	Just after third moult		3
			2	37

vestimenti. He says Hase ('15) studied the effect of lice on behalf of the war office in Germany. He questioned one thousand persons on the subject and was able to place them under four groups. First, *persons who are not attacked by lice;* second, those who are attacked continuously and are sensitive to bites; third, those who are suffering from bites became immune to the effects; fourth, those who have been bitten continuously, but do not suffer from the effects. In group No. 1, he mentions a man who remained untouched by lice, but suffered from fleas. In group No. 2, a man who suffered from both lice and fleas. In group No. 3, a man who was bitten by lice but avoided by fleas.

It is frequently stated by people whose intelligence and accuracy of statement are beyond question that they are never attacked by a definite species of insect though surrounded by people who are infested by them. If such evidence is an accurate statement of facts, such facts merit careful scientific experiment not only to demonstrate their accuracy, but to search for the cause of the immunity. In the case of *Pediculus vestimenti* the practical value of such investigations is self-evident.

In June, 1919, I met in Soissons a very intelligent soldier who is convinced that *Pediculus vestimenti* will not attack him. Sergeant Du R., of Ohio, age 28, has been in France two years, was many months at the front and frequently exposed to the pest. He slept with his brother from February 15 to May 5, 1917, and during that time his brother was infested with *Pediculus vesti-*

menti and suffered greatly from the infestation. Du R. says he was never attacked at any time during that period and later he slept for two weeks between two soldiers who were infested and the lice were also in the blankets. During those two weeks he had no lice whatever, though he frequently searched for them. He kindly consented to allow me to experiment with his case and July 19, 1919, I took three young lice to Soissons for a preliminary test. My aim was to determine whether their behavior showed any abnormal features while they were on Du R.'s skin. At nine p.m. they were fed on a normal host and at ten p.m. they were placed on Du R.'s arm. Feeding one hour before the test eliminated the factor of intense hunger which might force them to bite a possibly distasteful host.

Result.—I could detect no abnormal features in the feeding. They bit almost at once and each fed at least twice during the fifteen minutes they were on Du R.'s arm. They were not restless, made no effort to escape, and were not combative.

Later this experiment was continued to determine whether the lice are not normally nourished by Du R.'s blood, whether biting is followed by itching, and whether selecting various areas on the arm would show that Du R.'s skin is abnormally thick. In my experience lice cannot feed where the skin is callous, for example, the distal end of the thumb or various places on the palm of the hand.

One naturally suspects that at least some cases of so-called immunity may be due solely to the fact that biting is not followed by itching. A case in point is that of a friend whose maid was infested with fleas, and Madame de B. expressed surprise that they had not attacked her also. Her maid replied "Madame is mistaken, for I found twelve on her underwear." This led to the discovery that she was bitten without being poisoned.

The following September, Sergeant Du R. came to the laboratory one hour daily for ten consecutive days and the lice were fed on various parts of his arm from the wrist to the shoulder. Those that died were dissected and their sex noted.

It was hoped these further experiments would conclusively answer the above mentioned three questions:

First—Is Du R.'s blood injurious to lice?

Second—Is biting followed by itching?
Third—Is there evidence that Du R.'s skin is abnormally thick?

Daily Record of the Experiment.

September 10: 33 lice were fed on the arm between the wrist and elbow. The lice varied in age, the older were between the second and third moult, and the younger before the first moult.

Behavior while Feeding.—All but one fed normally, showing no effort to escape, though they were slow in commencing to bite. The one that refused to bite Du R. was fed on a normal host four hours later and bit at once.

September 11: Three dead (all ♂♂). Added 3 just hatched (total 33).

Behavior while Feeding.—The 3 just hatched bit at once and fed normally. One of the older lot did not bite, for the digestive tube was ruptured. It was killed and dissected (a ♀). The remaining 29 fed normally.

September 12: Four dead. Three of the four died while moulting (3 ♂♂ and 1 ♀).

Behavior while Feeding.—One of the three that were hatched September 11 took scarcely any blood, the other two did not bite for fifteen minutes and then fed normally. One of the older lot did not bite, having moulted abnormally. The balance fed normally. The one that could not bite was killed and dissected (a ♀).

September 13: Two dead (both ♂♂).

Behavior while Feeding.—One of the 3 that were hatched September 11 did not bite and died a few hours later (a ♂). One of the older lot did not bite; it was therefore killed (a ♀).

The balance fed normally.

September 14: Three dead—one while moulting and one immediately after the second moult (2 ♂♂ and 1 ♀).

Behavior while Feeding.—One of the 2 hatched September 11 took very little blood and the other died on Du R.'s arm (a ♀).

The balance fed normally.

September 15: One dead. Died while moulting (a ♂).

Behavior while Feeding.—All fed normally.

September 16: Three dead before the second moult (1 ♂ and

2 ♀♀). They are full of blood and apparently normal, and this evidence that the blood is not digested is apparent in nearly all the lice that have died.

Behavior while Feeding.—All fed normally.

September 17: One dead (a ♂). He was full of blood and apparently normal.

Behavior while Feeding.—The older lot fed quietly but took scarcely any blood. The one hatched September 11 was very restless and took no blood.

September 18: Two dead (both ♂♂). They were full of blood and apparently normal.

Behavior while Feeding.—The older lot fed normally. The one hatched September 11 ran around for 45 minutes and then fed normally.

September 19: The one hatched September 11 is dead (a ♂). He had digested some of the blood taken yesterday and appeared normal.

Behavior while Feeding.—One did not bite, the balance fed normally.

Results.

The death rate for the ten days feeding is certainly abnormally high. Of the 36 used for the experiment, 26 died and as most of these died before the second moult, and several died while moulting, the facts seem to indicate that Du R.'s blood does not nourish the lice normally but it does not warrant assuming that it adequately explains his apparent immunity. The proportion of the sexes of those that died was 17 ♂♂ and 9 ♀♀.

The second question—"Is biting followed by itching?"—can be answered conclusively, for the itching was absolutely normal and very distressing. This proves that his immunity was not a case of being deceived by the fact that biting was not followed by itching.

The third question—"Is there evidence that Du R.'s skin is abnormally thick?"—can also be answered conclusively. The lice had no difficulty in drawing blood and even the nymphs just emerged from the shell, bit at once and fed normally. The lice were fed on various areas of the arm from the wrist to the shoulder and showed no evidence of any abnormal effort in getting the blood.

A few smear preparations were made of the blood from the digestive tube; but showed no evidence of infection. The fact that nearly all the lice that died were full of undigested blood is the only evidence that Du R.'s blood is not normal.

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THE FERTILIZATION-REACTION IN ECHINARACHNIUS PARMA.

IV. A Further Analysis of the Nature of Butyric Acid Activation.

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I. Introduction.

In a previous communication (Just, '19*c*) the writer has shown that the butyric acid activation of *Echinarachnius* eggs as shown by membrane lifting is complete since these eggs (following insemination) cannot be fertilized with sperm even after removal of the membranes. With membrane lifting, therefore, the eggs undergo an irreversible change. Those eggs, however, which following exposure to butyric acid at or below the optimum length of time for perfect membranes fail to show membranes are not activated because they are still capable of complete fertilization yielding on insemination normal membranes, cleavage, and larvæ. Likewise, eggs which have had an exposure to butyric acid beyond the optimum for membrane lifting, and which show thick jelly-like cortices without the wide perivitelline space so characteristic of both inseminated and butyric acid activated eggs, are still capable of fertilizaton although their development subsequent to insemination is abnormal. This response to insemination of these three classes of butyric treated eggs—activated, exposed but not activated, and over-exposed—constitutes a criterion in the analysis of activation as striking as the visible structural changes in the cortex of inseminated eggs which are the criterion of normal sperm activation. It seemed, with the aid of this criterion, desirable to attempt a further analysis of the activation process in *Echinarachnius,* and to this end the writer has made observations, herewith reported, on the duration of the fertilization capacity of butyric acid treated eggs. At the same time it was possible to study the rate of cytolysis of butyric acid treated eggs in sea-water as compared with normal eggs in sea-water. The results of these two lines of observations on butyric acid treated eggs give a possible explanation of the nature of butyric acid activation in *Echinarachnius* which involves the whole theory of activation. Following, therefore, the presentation of the observations on cytolysis (Part II.) and those on the duration of fertilization capacity (Part III.) is a general discussion of these results and their bearing on the theory of activation (Part IV.).

II. Observations on the Cytolysis of Butyric Acid Treated Eggs.

Now, it is a well-known fact that marine ova normally shed in sea-water when physiologically "ripe" for fertilization in sea-water cytolyze at a rate which varies with different species. Cytolysis of the uninseminated egg in normal sea-water is a natural phenomenon. Thus, according to Goldfarb:

"The consummation of the various deteriorating changes in ageing eggs is cytolysis and death.

"Cytolysis of sea-urchin eggs under the influence of various experimental conditions, such as saponin, salycyl, aldehydes, propyl alcohol, distilled water, etc., have been carefully described by Loeb. Loeb speaks of two methods of cytolysis, which he calls 'white' and 'black.'

"Ageing eggs cytolyze in the same two ways. In the 'white' or cytolysis by liquefaction, a changed permeability of the cortical layer permits an increasing volume of sea-water to enter the egg with a corresponding enlargement of the egg, a more viscous condition of the cytoplasm, a diminution in size of the protoplasmic granules, and an increasingly hyaline and translucent appearance of the egg. In the second type of cytolysis, there is either far less increase in size of the egg or no increase at all; the central mass remains opaque and becomes increasingly opaque; the cytoplasm is far less viscous; the outer surface which is hyaline is fragmented, and sometimes the inner mass as well, is fragmented, and the outer fragments fall off, with a consequent diminution in size of the eggs, even far below the normal.

"I was unable satisfactorily to establish whether these two are independent methods of cytolysis, possibly associated with different degrees of virility of the eggs or whether they are sequential phenomena. In most cultures, both types of cytolysis are seen at the same time.

"The onset differs in the eggs of different females, and, as in other evidences of ageing, this variation is due to differences in the physiologic condition of the eggs at liberation. Those eggs which were in good physiologic condition at liberation, cytolyzed late; those in poor condition, early. Eggs in relatively similar physiologic condition cytolyzed at a similar rate at the same temperature. The greater the temperature, the greater the rate.

"In *Toxopneustes,* cytolysis in any considerable numbers was first observed when ⅕ hour old in experiment 1, ⅕ hour old in experiment 3, 6 hours old in experiment 2 and 5, 11 hours old in experiment 4, 20 hours old in experiment 9. In *Hipponoe,* the rate of cytolysis is essentially the same as in *Toxopneustes.*

"In *Arbacia* it was much slower. Beginning, in the given experimental conditions, in about 28 hours as in experiment 17, and extending to 42 hours as in series 19, 46 hours in experiment 18, and later in other experiments. This difference in rate of cytolysis is in part due to differences in temperature of the sea-water in the two localities, but it is also due, and is another evidence of, a protoplasmic difference in the two species of eggs."

Now, the longevity of the egg in sea-water depends upon the temperature, the physiological condition of the egg when shed—as determined by its size and presence of the jelly hull—the time in the breeding season, and the bulk of the eggs (that is, their concentration in a given volume of sea-water). To make comparisons, therefore, Goldfarb took precautions especially with reference to the last-mentioned factor, using as near as possible the same concentration of egg suspension. Nothing is clearer from these observations of his than the fact that eggs which are normally fertilized in sea-water after coming into sea-water and failing of insemination gradually die; for the cytolyzing egg is a moribund egg. Since it was not my intention to investigate the effect of various factors on this sea-water cytolysis—and Goldfarb's work made such an investigation unnecessary—I gave no particular attention to them in my observations. All that I wished to do in my study was to compare the rate of cytolysis in sea-water of eggs previously unexposed to butyric acid with those eggs in sea-water previously exposed for varying lengths of time to the action of this acid. My observations brought out a highly interesting fact; namely, that eggs under-exposed to butyric acid (*i.e.,* eggs that formed no membranes after transferral to normal sea-water) cytolyze at a slower rate than unexposed eggs. In other words, underexposure to butyric acid is beneficial to eggs in delaying the normal cytolytic effect of sea-water. To be sure, the factors temperature, seasonal variation, physiological condition of the ova, etc., play a part in the process; but, obviously, in any given observation in which a comparison is made between sea-water cytolysis and butyric acid cytolysis, since the eggs were from the same female and were approximately of equal mass in equal volume of water in the various dishes, the conditions were uniform.

Briefly, I found that if eggs of the sand dollar, *Echinarachnius parma,* after exposure to a mixture of *n*/10 butyric acid and sea-water (in the proportions 2 c.c. of acid plus 50 c.c. of sea-water) be transferred at varying intervals up to two or three minutes to dishes of pure clean sea-water they are cytolyzed during the ensuing thirty-six to forty-eight hours to complete disintegration. The rate of cytolysis—such factors as temperature, physiological condition of the eggs, seasonal variation, and mass of eggs to the amount of sea-water used being taken into account—is determined by the length of the exposure to the butyric acid sea-water: (1) Eggs which have been exposed for one minute or more designated "over-exposed eggs" because they have remained in the butyric acid mixture beyond the length of time for best membranes cytolyze most rapidly; (2) eggs exposed under the optimum time for perfect membranes which are designated "under-exposed eggs" cytolyze more slowly; and (3) eggs which have had the optimum exposure (around 35 seconds) for perfect membranes cytolyze at a rate which is intermediate of the "over-" and "under-exposed" eggs.

A. The Method.

The procedure is as follows: Eggs shed into perfectly clean dry watch glasses or dry eggs washed off the ovaries as they exude or concentrated washed eggs are gathered up in a pipette. A tenth or twelfth of these eggs is set aside as a control in normal sea-water, the remainder exposed to 10 c.c. of butyric acid sea-water. From the butyric acid sea-water after 10, 15, 20, 25, 30, 35, 40, 50, 60, 90 and 120 seconds equal portions of the suspension are carried over to dishes containing 250 c.c. of sea-water. The control eggs are then removed to a dish of 250 c.c. of sea-water. Thus, a series of dishes containing approximately equal masses of eggs is made. Citations from the observations follow.

B. The Observations.

The following protocols, the first of the thirty 1919 observations and confirmatory of others previously made, are in no wise selected. They are given because they are simpler to present than some of the later which are bound up with other experiments of a different category—the presentation of which might draw attention from the salient feature which is under consideration.

Experiment 2 B. June 14, 8:00 A.M. Eggs taken from a very good female as they exude from the ovaries. Control in sea-water, remainder placed in 10 c.c. butyric acid sea-water from which portions removed to 250 c.c. of clean sea-water at intervals of 10, 15, 20, 25, 30, 35, 40, 50, 60, 90, and 120 seconds.

June 14, 3:00 P.M. Eggs examined and counts made for cytolyzing eggs with the following results:

	No.											
	1	2	3	4	5	6	7	8	9	10	11	12
Exposure to butyric acid in seconds.......	0	10	15	20	25	30	35	40	50	60	90	120
Per cent. of cytolyzing eggs	50	20	15	20	50	85	75	94	95	100	100	100

In this observation we find half the eggs in sea-water cytolyzed seven hours after removal from the female. Eggs treated with butyric acid for ten to twenty seconds fare better in sea-water; and eggs exposed for the optimum time while cytolyzing in high per cent. at this time still do not show complete cytolysis as do the over-exposed groups.

Experiment 6 B. June 15, 3:00 P.M. Eggs from one female shaken off ovaries into 10 c.c. of butyric acid sea-water; portions transferred to dishes each with 250 c.c. of sea-water after 15–120 seconds as shown below. Control in sea-water. Membranes best after the 35 and 40 seconds exposures, 93 and 90 per cent. respectively. A few perfect membranes after 60 seconds exposure, others contracted membranes, 90 and 120 seconds exposures give 100 per cent. contracted membranes.

June 16, 8:00 A.M. Dishes examined with the following counts shown:

	No.											
	1	2	3	4	5	6	7	8	9	10	11	12
Exposure to butyric acid in seconds..........	0	10	15	20	25	30	35	40	50	60	90	120
Per cent. of cytolyzing eggs.....	60	—	40	25	50	65	90	100	100	100	100	—

Seventeen hours after treatment with butyric a hundred per cent. cytolysis is shown in the over-exposed eggs. The under-exposed eggs cytolyze at a lesser rate than the control in sea-water.

Experiment 7 B. June 16, 7:00 P.M. Dry washed eggs from one female exposed to butyric acid up to 120 seconds (Nos. 2–11). Control (No. 1 in sea-water). Very few membranes after 35 and 40 seconds exposures. 60, 90, and 120 seconds exposures give 100 per cent. contracted membranes.

June 17, 8:00 A.M. Eggs from dishes show cytolysis as follows:

	No.											
	1	2	3	4	5	6	7	8	9	10	11	12
Exposure to butyric acid in seconds	0	10	15	20	25	30	35	40	50	60	90	120
Per cent. of cytolyzing eggs	10	—	24	36	41	90	94	100	99	100	100	100

This observation on washed eggs shows very clearly that over-exposed eggs thirteen hours after treatment are cytolyzed to a greater extent than under-exposed. The control eggs cytolyze more slowly than the under-exposed eggs which is an exception to the rule.

Experiment 8 B. June 17. Eggs from a very good female exposed to butyric acid sea-water from 15 to 120 seconds. Control in normal sea-water show some eggs devoid of jelly. Four hours later these eggs show per cent. cytolyzing as follows:

	No.											
	1	2	3	4	5	6	7	8	9	10	11	12
Exposure to butyric acid in seconds	0	10	15	20	25	30	35	40	50	60	90	120
Per cent. of cytolyzing eggs	50	—	29	21	74	80	86	85	50	100	100	—

Four hours after butyric acid treatment we find over-exposed eggs 100 per cent. cytolyzed. In most cases this percentage is reached after three hours. Cytolysis begins in these eggs within an hour after treatment when most of the other eggs including the control are intact. The beneficial effects of short exposure to butyric acid are strikingly brought out in this observation.

Experiment 9 B. June 19, 7:00 P.M. Eggs treated for 0, 15, 20, 25, 35, 40, 60, 90, and 120 seconds.

June 20, 8:00 A.M. These eggs after thirteen hours show the following:

	No.											
	1	2	3	4	5	6	7	8	9	10	11	12
Exposure to butyric acid in seconds	0	10	15	20	25	30	35	40	50	60	90	120
Per cent. of cytolyzing eggs	26	—	10	24	18	—	42	40	—	100	100	100

This observation demonstrates that while every egg of the over-exposed dishes is in cytolysis more than half the eggs of the 35 and 40 seconds exposures are intact and perfect in appearance. The large body of data universally shows that though cytolysis in over-exposed eggs may begin within an hour after exposure, the case is different for the eggs with membranes which are largely intact several hours after exposure. The membrane formation in these eggs is not therefore *per se* the cause of cytolysis. Moreover, here again as in other cases cited we note the beneficial effect of short exposure to butyric acid in arresting the normal cytolytic action of sea-water.

Experiment 10 B. June 24, 9:00 A.M. Eggs exposed to butyric acid sea-water from 15 to 120 seconds. Control in sea-water. At 3:30 P.M.—6½ hours after treatment—eggs examined for cytolysis. Another examination at 8:30 A.M., June 25. The counts of these two examinations are as follows:

	No.											
	1	2	3	4	5	6	7	8	9	10	11	12
Exposure to butyric acid in seconds	0	10	15	20	25	30	35	40	50	60	90	120
Per cent. of cytolyzing eggs after 6½ hours	24	—	2	12	36	50	76	30	—	60	80	100
Per cent. of cytolyzing eggs after 23½ hours	11	—	3	23	60	81	84	100	—	100	100	100

Comparison of the percentages of cytolyzing eggs six and one half hours and twenty-three and one half hours after treatment with butyric acid clearly shows that the cytolysis of eggs exposed for the optimum length of time is of a different order from that of over-exposed eggs. This was brought out repeatedly in the observations. Over-exposed eggs cytolyze rapidly *because* they are over-exposed; eggs with membranes cytolyze despite the fact that they have membranes.

Experiment 13 B. June 25, 11:20 A.M. Eggs from one female washed in 10 c.c. of sea-water twice in ten minutes. Sample removed to normal sea-water as control (No. 1); remaining eggs exposed to butyric acid sea-water for 15, 20, 25, 30, 35, 40, 60, 90, and 120 seconds.

June 26, 9:40 to 10:20 A.M. Eggs from each lot examined for cytolysis with the following counts:

	No.									
	1	2	3	4	5	6	7	8	9	10
Exposure to butyric acid in seconds	0	15	20	25	30	35	40	60	90	120
Per cent. of cytolyzing eggs	36	19	22	39	68	96	100	100	100	100

About twenty-three hours after treatment eggs of both the optimum (between 35 and 40 seconds exposure) and the over-exposed groups show high percentages of cytolysis. This result, however, should not be taken alone for as shown in other observations over-exposed eggs early show 100 per cent. cytolysis while optimum and under-exposed eggs are still intact.

If we tabulate the figures given in these seven protocols one may see at a glance that the shorter exposures to butyric acid are markedly beneficial in protecting the egg against the normal cytolytic action of sea-water.

TABLE I.

	No. of Dish.											
	(Control) 1	2	3	4	5	6	7	8	9	10	11	12
Exposure to butyric acid in seconds	0	10	15	20	25	30	35	40	50	60	90	120
Per cent. of cytolysis 7 hours after. Ex. 2 B	50	20	15	20	50	85	75	94	95	100	100	100
Per cent. of cytolysis 17 hours after. Ex. 6 B	60	—	40	25	50	65	90	100	100	100	100	—
Per cent. of cytolysis 13 hours after. Ex. 7 B	10	—	24	36	41	90	94	100	99	100	100	100
Per cent. of cytolysis 4 hours after. Ex. 8 B	50	—	29	21	74	80	86	85	50	100	100	—
Per cent. of cytolysis 13 hours after. Ex. 9 B	26	—	10	24	18	—	42	40	—	100	100	100
Per cent. of cytolysis $6\frac{1}{2}$ hours after. Ex. 10 B	24	—	2	12	36	50	76	30	—	60	80	100
Per cent. of cytolysis 23 hours after. Ex. 13 B	36	—	19	27	39	68	96	100	—	100	100	100

C. Discussion.

In the original observations the eggs were exposed to *one* c.c. of *n*/10 butyric acid plus fifty c.c. of sea-water for twenty seconds. Struck with the remarkable longevity of these eggs on return to normal sea-water as evidenced by their low per cent. of cytolysis and their retention of fertilization capacity as compared with the control in sea-water, the writer was led to continue the observations during succeeding summers using also the mixture *two* c.c. of $n/10$ butyric acid plus fifty c.c. of sea-water. Practically, if the egg does not form a membrane on removal from butyric acid, it gains through this acid treatment a resistance to the normal cytolytic action of the sea-water.

Examination of the tabulated data likewise clearly reveals that eggs exposed above one minute to the concentration of butyric acid employed are seriously injured. The individual observations as cited do not, however, so clearly show that these over-exposed eggs very early after removal from the butyric acid exhibit marked cytolytic changes. And for this reason: What primarily the writer aimed to determine was the point in time after removal from sea-water when one hundred per cent. of the *under-exposed* eggs (*i.e.*, eggs that show no membranes) completely disintegrate—a point by no means easy to fix because of the immunity against cytolysis conferred by the acid treatment. It may be recalled, nevertheless, that attention has been directed to the fact that cytolysis in the over-exposed egg begins very early. In general, over-exposed eggs showing one hundred per cent. thickened cortices give one hundred per cent. cytolysis within two hours after treatment. This fact was clearly revealed early in the study of the effect of butyric acid on these eggs. Another characteristic of over-exposed eggs as elsewhere noted (Just, '19*c*) is their response to shaking: the jelly-like cortex breaks and the eggs give off buds. That is, gentle shaking hastens cytolysis. In the egg with butyric acid membrane, the same or even more vigorous shaking produces merely a collapse of the membrane without injury to the vitellus. We must interpret these facts as meaning that the over-exposed egg is an egg in the initial stages of cytolysis the visible manifestation of which is the swollen cortex that so sharply differentiates it from the optimum exposed egg with its full round membrane and wide perivitelline space. But this interpretation by no means needs rest on the findings in *Echinarachnius* alone.

In the first place, Loeb has recorded similar observations for speaking of *Arbacia* he says: "Since the membrane called forth by butyric acid is not always plainly visible, it is a prerequisite that always one set of such eggs should be set aside as controls to ascertain whether or not all the eggs disintegrate rapidly (if no second treatment is given them). Only if they all disintegrate rapidly have we any guarantee that in all of them the membrane formation has been effective" (Loeb, '15, page 262). Now, curiously enough, Loeb never saw membrane formation in *Arbacia* for he tells us of this egg: "When transferred to sea-water, they did not form a conspicuous fertilization membrane as did the eggs of *S. purpuratus* under the same circumstances, but only a fine gelatinous layer which was not easily visible" (Loeb, '13, page 71). Since, however, one can with proper exposure to butyric acid obtain very beautiful membranes in *Arbacia* and can obtain "the fine gelatinous layer" only through over-exposure it is clear that Loeb was dealing with over-exposed eggs which tend to disintegrate rapidly. Thus, according to Loeb over-exposure hastens cytolysis in *Arbacia*.

Again, Moore, working likewise with *Arbacia,* states: "Eggs exposed to butyric acid for slightly longer than the optimum time for membrane production do not produce membranes yet they cytolyze even more rapidly than in cases where membranes have been produced . . . they will many times have almost completely disintegrated when the first signs of cytolysis appear in eggs provided with membranes" (Moore, '16). Here, again, is noted a sharp difference between properly exposed and over-exposed *Arbacia* eggs.

Finally, Herlant ('17) working with *Paracentrotus* makes the point that it is not the formation of membranes that brings about cytolysis, for these eggs with butyric acid membranes may withstand the action of sea-water for twelve to fifteen hours before the onset of cytolysis. The egg cytolyzes despite the presence of the membrane because of internal changes due to nuclear activity.

In conclusion, then, we find that these facts taken together indicate that there is a clear cut difference between the butyric acid treated egg with a membrane and the over-exposed egg with a jelly-like cortex. The over-exposed egg is an egg in the initial stages of cytolysis, its jelly-like cortex marks the beginning of cytolysis. But it is fallacious to argue that because the over-exposed egg is a cytolyzing egg the properly exposed egg, because of its membrane, is likewise a cytolyzing egg. That it cytolyzes is true, but it is also true that the normal uninseminated egg in normal sea-water cytolyzes. Instead, we might more logically contend that since under-exposed eggs are resistant to cytolysis that *membrane formation protects against cytolysis* for the under-exposed egg is no farther below the proper exposure for membrane production than the over-exposed egg is above it.

III. Observations on the Duration of Fertilization Capacity in Butyric Acid Treated Eggs.

In *Echinarachnius,* membrane elevation is a sign of complete activation whether the elevation is due to the agency of sperm or of proper butyric acid treatment for in either case the egg does not fertilize even after removal of the membrane. Activation is an irreversible reaction. But, neither the under-exposed egg that forms no membrane nor the over-exposed egg that likewise forms no membrane but exhibits a jelly-like cortex is activated since they are both capable of insemination; the former producing normal membrane, cleavage, and larva; the latter no membrane, abnormal cleavage, and larva. Thus, the response to insemination constitutes a physiological criterion of activation no less important than the striking morphological cortical changes. Since, now, we know that in *Echinarachnius* the effect of membrane production by butyric acid is the instant loss of fertilization capacity (Just, '19*c*) it would seem to be important in the analysis of the nature of butyric acid activation to know the duration of the fertilization capacity of under-exposed and over-exposed eggs. This section presents observations on this point which prove that, as in the case of the rate of cytolysis, there exist sharply defined differences among the three classes of eggs: under-, over-exposed, and activated.

A. The Method.

The method used in these observations is very simple. Following their exposure to butyric acid for varying lengths of time eggs are transferred to dishes of 250 c.c. of sea-water from which samples are removed at intervals and inseminated with fresh sperm. Control eggs of bulk approximately equal to any of the exposed lots are similarly inseminated. To save time and material the eggs used for the study of cytolysis were frequently used for this set of observations. Every precaution was exercised to keep everything clean and sterile; likewise to procure conditions as uniform as possible.

B. The Observations.

The observations here presented are chosen for purposes of comparison from those that have already been reported in the section on cytolysis. And we may say at the outset that all these data show that the duration of fertilization capacity of the under- and the over-exposed eggs runs parallel with resistance to cytolysis. Under-exposure, then, not only protects against cytolysis but preserves the fertilization capacity; while over-exposure which hastens cytolysis likewise shortens the period after exposure during which the eggs will fertilize—their failure to fertilize constitutes the best index of initial cytolysis.

Experiment 2 B. June 14, 8:00 A.M. Eggs exposed to butyric acid for varying lengths of time then transferred to 250 c.c. of sea-water. Control in sea-water (see page 284).

12:02 P.M. Samples from dishes Nos. 1 (control) and 2–12 inclusive inseminated with fresh sperm suspension.

1:50 P.M. Cleavages in these dishes of inseminated eggs counted and percentages recorded as follows:

	No.											
	1	2	3	4	5	6	7	8	9	10	11	12
Exposure to butyric acid in seconds	0	10	15	20	25	30	35	40	50	60	90	120
Per cent. of cleavage	17	66	12	40	7	0	3	0	0	0	0	0

Here eggs inseminated about four hours after exposure show no cleavage in the over-exposed lots while the percentage of cleavage in the under-exposed lots is high—much higher in the case of the ten second exposure than in the control. Eggs with optimum treatment which form highest per cent. of membranes show no cleavage. There is a difference, however, between these eggs with membranes and the over-exposed eggs; for, as previously shown (Just, '19*c*), eggs with membranes the instant of membrane elevation are incapable of fertilization; on the other hand, the over-exposed egg is fertilized on removal to sea-water. Its capacity for fertilization endures for one hour or more.

Experiment 6 B. June 16, 1:30 P.M. Eggs of Experiment 6 B whose percentages of cytolysis noted at 8:00 A.M. (see page 285) inseminated at 1:30 P.M. Cleavage counts at 4:30 P.M. follow:

	No.										
	1	2	3	4	5	6	7	8	9	10	11
Exposure to butyric acid	0	10	15	20	25	30	35	40	50	60	90
Per cent. of cleavage	0		75	15	10	3	1	0	0	0	0

Eggs we note that received a 15-second exposure show twenty-two and one half hours afterward 75 per cent. cleavages whereas normal eggs which have been in normal sea-water for the same length of time have lost completely their capacity for fertilization. This is by no means a solitary finding; the acid treatment markedly benefits the egg in protecting it against loss of fertilizing power. Activated eggs and over-exposed eggs have lost their capacity for fertilization.

Experiment 8 B. June 17, 8:35 A.M. 13 hours after exposure to butyric acid for 0, 15, 20, 25, 30, 35, 40, 50, 60, 120 seconds, eggs inseminated with fresh sperm suspension.

12:00 M. No cleavage in any dish.

These eggs lost their fertilization capacity relatively early.

Experiment 13 B. June 25, 5:50 P.M. Eggs from each of the dishes of the series exposed at 11:20 together with control (No. 1) inseminated with fresh sperm suspension.

7:30 P.M. Good cleavages with membranes up to and including No. 5. Best cleavages as to per cent., form, and size in Nos. 2, 3, and 4. Dishes set aside overnight.

June 26, 8:30 A.M. "Swimmers" counted in dishes, per cent. noted as follows:

	No.									
	1	2	3	4	5	6	7	8	9	10
Exposure to butyric acid in seconds....	0	15	20	25	30	35	40	60	90	120
Per cent. of "swimmers"......	31	76	62	71	38	0	0	0	0	0

This observation is cited to show that the stale egg previously treated with butyric acid is capable of developing into larvæ. No. 1 gave good larvæ; Nos. 2 and 3 gave normal larvæ; Nos. 4 and 5 were poor, large number of exogastrulæ.

July 1. Six determinations on different lots of eggs showed that eggs having 120 seconds exposure lost completely their fertilization capacity in 43, 61, 54, 73, 78, and 51 minutes respectively. Not a single egg had a membrane, but all had the jelly-like cortex. In each case, the 35 second exposure showed over 90 per cent. of eggs intact; the under-exposed eggs were all intact. The controls of the six lots showed 16, 19, 7, 14, 9, 3 per cent. cytolysis respectively.

C. Discussion.

The foregoing data constitute the evidence for the conclusion that short exposure to 2 c.c. of $n/10$ butyric acid plus 50 c.c. of sea-water prolongs the capacity of the egg to respond more or less normally to insemination. Compared with the normal egg in normal sea-water, the egg which treated with butyric acid fails to form a membrane not only is endowed with certain protection against the cytolytic action of sea-water but also is restrained from a too rapid loss of fertilizing power. This effect as in the case of cytolysis may be produced with one c.c. of $n/10$ butyric acid plus 50 c.c. of sea-water. Indeed, the consequence of the treatment with butyric acid may be an actual improvement of the normal egg as shown by the size, vigor, and longevity of the larva. Thus, eggs treated for ten seconds with the higher concentration of acid or for twenty seconds with the lower after twenty-four hours in sea-water show one hundred per cent. intact without any sign of cytolysis, whereas control eggs from the same female may show from fifteen to one hundred per cent. of cytolysis—observations frequently made during three seasons. Such eggs on insemination yield close to one hundred per cent.

fertilization but the control eggs may yield no fertilization. Freshly liberated eggs may be and often are inferior to these butyric acid treated eggs: they vary in size, in regularity of cleavage, and in vigor of larvæ.

The normally shed eggs of *Echinarachnius* allowed to stand in sea-water exhibit certain changes such as loss of jelly, change in size, etc., to complete cytolysis the beginning of which is the swelling of the cortex. If during the interval between shedding and complete disintegration—the time of which varies with temperature and with different lots of eggs—we inseminate these staling eggs periodically, we obtain additional evidence of physiological deterioration. For the uninseminated eggs of *Echinarachnius* the sea-water has an injurious action which manifests itself subsequent to insemination through the inability of the eggs to produce membranes, the loosening of blastomeres during cleavage, the production of aberrant larval forms, etc. (cf. Goldfarb's studies). Against this cytolytic effect of sea-water short treatment with butyric acid protects, the eggs cytolyzing at a slower rate and retaining fertilizing power for a longer period. It is only after several hours that these butyric acid treated eggs fail to respond to insemination.

Although the short exposure to butyric acid is thus beneficial in conserving fertilization power, longer exposures are decidedly harmful. An exposure of ninety seconds, for example, may be sufficient to bring about the complete loss of fertilizability within an hour. Such an exposure is beyond the optimum for activation and does not result in activation because the activated egg, which is the egg with full membrane, loses instantly with activation its capacity for fertilization. Over-exposure is comparable to prolonged staling in sea-water inasmuch as the effect of either is first to destroy the membrane, but the activable substances remain, albeit the long exposure has injured the egg, as evidenced through its jelly-like cortex, and the egg develops on insemination without membrane lifting. Failing of insemination the over-exposed egg soon loses its fertilization capacity because of the onset of death changes that manifest themselves in the thickening of the cortex which is the beginning of cytolysis.

In conclusion, these observations on the duration of the fertilization capacity of butyric acid treated eggs show three physiologically distinct classes: under-exposed inactivated eggs whose fertilizability the acid treatment prolongs; activated eggs, with membranes, rendered incapable of fertilization at the instant of activation; and over-exposed inactivated eggs whose capacity for fertilization is cut short by the excessive action of the acid which initiates destructive changes.

IV. General Discussion.

1. The activation of the egg whether produced through sperm or butyric acid renders the egg incapable of insemination. Thus, Moore found *Arbacia* eggs following the successful production of membranes with butyric acid refractory to fertilization. In *Echinarachnius* (Just, '19*c*) subsequent to membrane formation with butyric acid the egg cannot be fertilized though the membrane be removed as soon as formed. Likewise, sperm activation renders the egg immune to the entry of any other sperm. The test, therefore, of complete activation is the response to insemination.

2. In *Echinarachnius* one may readily follow the changes leading to membrane production consequent on insemination: In the cortex beginning at the site of sperm entry droplets escaping push the membrane off. These droplets are discrete bodies that squeezed out of the cortex may cross the perivitelline space and reach the membrane before they go into solution. In other words, the *underlying process of membrane formation is a secretion or liquefaction of the cortex*. The result of this liquefaction is the wide perivitelline space and the diminution in the size of the egg. This process easily followed in *Echinarachnius* lends support to Loeb's suspicion that "the membrane formation is the result of a process of secretion of a liquid from the egg" (Loeb, 13, page 216). It also suggests the cortical secretion in the eggs of *Nereis* and *Platynereis*.

While we may say that in *Echinarachnius* certainly membrane lifting is not the cause but rather the result of activation, at least in sperm activation since the egg becomes immune to the entry of any other sperm before the membrane lifts (Just, '19*a*), yet membrane lifting is an important easily visible sign of complete activation.

3. The activated egg is an egg that has lost its fertilizin. The evidence of Lillie, Moore, and Just on this point working with two species of echinids and with *Nereis* shows very clearly that once activated, as shown by membrane production, the egg ceases to secrete fertilizin. Apparently, this loss is coincident with activation representing a neutralization of fertilizin with antifertilizin in the egg cortex.

We find these results, then, in the fully activated egg: (1) inability to respond to insemination, (2) the presence of a membrane widely separated from the vitellus which has arisen as the result of a cortical liquefaction, and (3) the absence of fertilizin. In the case of the sperm activated egg, the normal cleavage and development follow insemination; but in the butyric activated egg without hypertonic treatment, after a longer or shorter residence in sea-water cytolysis and death result.

Comparison with the over-exposed egg yields many dissimilarities.

1. The over-exposed egg is capable of insemination. Though it would appear Loeb failed to get such eggs to fertilize, the observations of Herbst, Moore, and Just seem to show that for a time at least after exposure these eggs will respond to insemination, albeit development is far from normal.

2. These eggs never form membranes on insemination.

In my judgment this failure to form a membrane after insemination is due to the loss of the membrane or membrane substance through too long exposure to the acid. I interpret the transparent jelly-like peripheral layer of these eggs to be not a swollen membrane but the cortex itself which swells as the result of the injurious action of the acid after loss of the membrane. This cortical change is the most striking characteristic of the over-exposed egg. In the activated egg, the cortex liquefies, pushes the membrane off and so brings about the formation of the perivitelline space; but in the over-exposed egg this cortex thickens.

3. The over-exposed egg, an egg still capable of fertilization, secretes fertilizin. On this point the studies of Moore admit no doubt. The over-exposed egg is not an activated egg.

As with the butyric acid activated egg without hypertonic treatment the over-exposed egg cytolyzes. The rate of cytolysis, however, is so different that we must regard the process of different order in the two cases.

Over-exposure to butyric acid is cytolytic; hence, the egg rapidly dies, the cortical thickening is the beginning of cytolysis. In the case of the activated egg, cytolysis is entirely secondary; cytolysis takes place in spite of activation not because of it. Moreover, that which first cytolyzes in the over-exposed egg, namely the cortex, is absent in the activated egg because it has liquefied. Therefore, the cytolysis in the two eggs is qualitatively different.

For these reasons, therefore, we must conclude that *the over-exposed egg is a cytolyzing egg but that activation is not a cytolysis.*

But since according to Loeb all activation is caused by a "superficial" cytolysis we must examine further his widely accepted theory.

A. The Cytolysis Theory of Loeb.

In his book "Artificial Parthenogenesis and Fertilization," Loeb tells us: "The object of these experiments was the substitution of physico-chemical agencies for the mysterious complex 'living spermatozoön.'" "This book gives a survey of the methods by which the unfertilized egg can be caused to develop into an embryo and the conclusions which can be drawn concerning the mechanism by which the spermatozoön produces this effect. The theory which the author published in 1905 and 1906 that at least two factors are involved in this process, namely, one which brings about a change in the surface of the egg (the essential factor) and a second, corrective factor, seems to explain all the phenomena observed in the new territory and has proved a reliable guide." Thus Loeb despite his numerous other suggestions as to the cause of development quite definitely commits himself to the cytolysis theory that the egg either by the "mysterious complex living spermatozoön" or by the "substitution of physico-chemical agencies" develops through a change in its surface which he calls "superficial cytolysis." Against this theory of activation several potent objections may be raised.

The cytolysis theory is based on results obtained with over-exposed eggs. Loeb argues that because a toxic agent—like saponin, benzole, toluene, etc.—destroys the egg, its normal development or its artificial parthenogenesis is likewise a destructive (cytolytic) process solely because in many cases the toxic agent if allowed to act but a short time induces membrane formation; prolonged exposure being cytolytic, the shorter exposure which induces membrane formation must therefore be cytolytic. It would be tedious to cite the pages in which this argument appears. A single quotation will suffice. "We can therefore say briefly that *all hemolytic agencies effect the activation of the unfertilized egg and this activation consists in a cytolysis of the cortical layer of the eggs*." The italics are Loeb's.

Moreover, Loeb practically tells us, as pointed out above that with *Arbacia* he employed over-exposed eggs and also that he used as a criterion for activation the rapidity with which the death changes (cytolysis) set in after treatment. The proper exposure for *Arbacia* eggs as first determined by Heilbrunn is much shorter than that which Loeb used and gives beautiful full membranes instead of the jelly-like film of the over-exposed egg. This over-exposure, therefore, simulates neither the cortical changes induced by the "complex 'living spermatozoön'" nor the artificial membrane formed through properly timed exposure.

The cytolysis theory, in the next place, is based on the superficial resemblance of cytolyzing eggs to cleaving eggs. Loeb continually speaks of "cleavage" when he means disintegration. Thus, page 76, he figures the "slow disintegration of the egg of the sea urchin at low temperature" which "can reach the eight cell stage." Butyric acid does not call forth cleavage in these urchin eggs. Herlant records much the same experience. Loeb also states that "centrosomes and two astrospheres are formed and the nucleus divides." Again, in the experience of others (cf. Herlant, also Just) the egg treated with butyric acid alone does not go beyond the monaster stage.

Likewise, on purely logical grounds the cytolysis theory should be rejected. Influenced in large measure no doubt by the interest then prevailing in the field of hemolysis, Loeb sought to bring the explanation of his work on "artificial parthenogenesis" in line with these current researches. He therefore conceived "artificial parthenogenesis" as a process comparable to hemolysis and all hemolytic agents as artificial excitants of development. Since, therefore, fertilization is to be regarded as a sort of "artificial parthenogenesis" and according to Loeb formally explained by these experiments of his rather than by actual investigation of the fertilization process *per se,* the egg is fertilized by a sperm-borne lysin. Thus, on a mere assumption based on the similar action of toxic agents on blood cells and on ova he first explains "artificial parthenogenesis" and on this bases another assumption to explain fertilization. Now, of course the mammalian red blood corpuscle is *sensu stricto* not a cell: in the absence of a nucleus its metabolism is scarcely that of a normal cell, its anabolic activity must be limited, and its physical properties altered. While we lack convincing data for the estimation of the life of the erythrocyte we well know that it is on the way to destruction. Again, if we call to mind the various agents of hemolysis from the simplest, distilled water, to the most complex, snake venom or foreign sera, we must admit that they certainly do not normally occur in blood plasma. Hemolysis is artificial or pathological. Loeb compares the highly pathological process of hemolysis to the initiation of development of the animal egg —perhaps the most complex cell in the living world, richly endowed with synthetic power, a constructive mechanism of well organized hereditable qualities. On the one side we have the artificially induced disintegration of a piece of a cell, on the other the normal activation that initiates the development of the future metazoön. On purely logical grounds, therefore, the cytolysis theory is scarcely tenable.

If anything were wanting in the case against the cytolysis theory we could point out that for the sea-urchins of Loeb's experiments no single hemolytic agent acting alone induces development; they induce membrane formation or with longer exposure death. Eggs exposed to these so-called agents of development need for development (production of cell division and swimming larvæ) hypertonic treatment. It is the hypertonic seawater alone as Morgan long ago showed that induces cleavage. One would scarcely denominate hypertonic solution as hemolytic.

Finally, the cytolysis theory assumes that the cytolysis of the over-exposed egg is of the same nature as that of the egg which has had through a lower exposure a membrane formed. This is purely an arbitrary assumption. The over-exposed egg cytolyzes rapidly because it is injured; the egg with the membrane cytolyzes in spite of the membrane and at a slower rate. According to Herlant it is the failure to cleave that finally kills the membrane egg. The cytolysis in these two classes of eggs thus springs from different causes. Nor is this all. The thickening of the cortex of the over-exposed egg we may regard as the initial step in cytolysis—or, if you please, as the mark of the injury that hastens cytolysis. In the membrane egg, however, this cortex has broken down through a process of liquefaction or secretion which is the fundamental phenomenon in the cortical change of which membrane elevation is a sequel. Therefore, the cytolysis in these two kinds of eggs must differ not only in time but in the quality of substances that cytolyze.

For these capital reasons, then, added to the conclusion of the results on *Echinarachnius* any theory of activation that postulates the cause of the initiation of development as a superficial cytolysis is difficult of defence. The cytolysis theory of development has blocked the path of progress to an understanding of the fertilization problem.

B. The Rhythmical Changes of Cell Division.

Following successful insemination the egg divides. Cell division is thus a criterion of fertilization. Many of the phenomena, however, revealed by the egg subsequent to its insemination are to be regarded as belonging not to the fertilization-reaction but to the physiology of cell division. Among these we find rhythmical changes that parallel cell division. For example, we may cite those rhythmical changes in viscosity so carefully investigated by Heilbrunn. He correlates these changes with the appearance and growth of the mitotic spindle during the division cycle. Viscosity changes are, therefore, to be regarded as an index to cell division. They are manifestations of the rhythm of cell division and are in no wise peculiar to fertilization. Here too belong other changes in the egg which follow insemination, such as: increased oxidation, increased permeability, varying susceptibility to heat, cold, ether, lack of oxygen, KCN, etc. They constitute indicia of cell division regardless of fertilization. An egg following insemination shows increase in viscosity, permeability, oxidation, and susceptibility to KCN. But these increases are not the "cause" of activation; they are the expression of the beginning of the rhythm of cell division.

If this position be tenable, if indeed these so-called indicia of cell division be simply the expression of cell division should we not expect to find that there are not such great changes in permeability, oxidation, or susceptibility to KCN in the case of an egg inseminated while in a stage of mitosis? Thus, in the sea urchin egg, in which maturation is complete and the nucleus at rest at the time of insemination, we should expect to find great physical and chemical changes due to the initiation of cell-division; but in an egg like that of the starfish inseminated during maturation we should not get such great changes. *And this indeed is the case.*

Loeb ('13) found, for example, in the sea urchin egg "that immediately after fertilization the egg consumed five to seven times as much oxygen as before fertilization." But he found conditions entirely different in the starfish egg: "No noticeable increase in the rate of oxidation is caused in this egg through the entrance of the spermatozoön. This is intelligible from the fact that those oxidations which lead to nuclear division were already going on in the eggs at the time the spermatozoön entered." In other words, insemination in the starfish egg does not initiate division and so does not increase the rate of oxidation.

R. S. Lillie has studied the permeability changes in fertilized eggs of *Arbacia* and *Echinarachnius,* both of which show the same kind of permeability changes. Thus, he found that *Arbacia* eggs take up water several times more rapidly after fertilization than before; so great is this difference as shown by the increased volume of the fertilized eggs that fertilized and unfertilized eggs in the same dish of hypotonic sea-water are easily separated. As would be expected, unfertilized and fertilized eggs differ in their response to hypertonic sea-water; the fertilized eggs lose water much more rapidly. *Echinarachnius* eggs behave similarly *but the eggs of Asterias show practically no difference before and after fertilization.* These results indicate that permeability changes, like those of oxidation, are indicia of cell division.

Finally, Mathews has studied the effect of KCN on fertilized *Asterias* and *Arbacia* eggs. His findings on *Arbacia* confirm the earlier observations of Lyon on this egg. Mathews finds that the susceptibility of the starfish egg to KCN coincides with the development of the asters, while the period of immunity coincides with the retrogression of the asters. There is thus a rhythm of alternate increase and decrease of susceptibility to KCN which accompanies the rhythm of the mitotic process. The susceptibility to cyanide is, therefore, comparable to the rhythm of viscosity changes and to that of permeability.

With *Asterias* eggs, however, Mathews got unsatisfactory results. He could not discover in this egg the sharp periods of susceptibility to KCN discovered in the *Arbacia* egg. I venture the opinion that this result is due to the fact that in *Asterias* the maturation division is in process at the time of insemination. It would, therefore, be difficult if not impossible to obtain in the *Asterias* egg just after insemination a stage comparable to that in the *Arbacia* egg just after insemination. Indeed, at no time after insemination until the appearance of the cleavage asters could a fair comparison between these eggs be made.

In brief, these changes—of viscosity, oxidation, permeability, and susceptibility to KCN—are not changes incident to the fertilization-reaction *per se;* they are changes that are bound up with the problem of cell division. They are, therefore, in no wise peculiar to the fertilized egg. They cannot be regarded as explaining the activation of the egg.

C. The Fertilizin Theory of Lillie.

At present the best working hypothesis for the study of the fertilization-reaction is the fertilizin theory of Lillie. The fertilizin theory postulates that development is initiated through the ovogenous substance, fertilizin. The sperm activates the fertilizin and this reaction between sperm and fertilizin is the first step in the developmental processes in the egg. The activated fertilizin acts on both egg and sperm.

In the egg, as a result of the activation of fertilizin, cortical changes take place. These changes may be initiated through the mere attachment of the sperm to the egg membrane, as in *Nereis* and *Platynereis* where jelly secretion from the cortex takes place before the penetration of the sperm. In *Echinarachnius* cortical changes leading to the lifting of the membrane are induced by the sperm or by proper exposure to butyric acid.

Fertilizin likewise acts on the sperm; as Lillie puts it, the sperm itself needs to be fertilized. As a result of the union of the sperm with fertilizin, the sperm head is made to swell. If fertilizin be absent, as in immature eggs, in butyric acid membrane eggs (Moore) or in stale eggs, the sperm head does not swell. Spermatozoa may penetrate these three classes of eggs but they undergo no change nor do the eggs develop. We have thus two criteria of the sperm-fertilizin-egg reaction: the cortical changes in the egg and later the swelling of the sperm head. But even before this the reaction is complete. It is not a reversible destructive reaction in need of a "corrective factor" but a constructive, irreversible, *practically instantaneous reaction* setting in motion the whole train of events, with accompanying changes in oxidation, permeability, etc., leading to the cleavage of the egg.

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BIOLOGICAL BULLETIN

THE PHYSICAL EFFECT OF ANESTHETICS UPON LIVING PROTOPLASM.[1]

L. V. HEILBRUNN.

Claude Bernard and many others after him have pointed out that anesthesia is a phenomenon common to all animals and plants. Vital processes of the most diverse types can be checked without destroying life itself.

Because of the general nature of anesthesia and because in many cases the same substances in not very different concentrations anesthetize widely different forms it has usually been felt that anesthetics act primarily upon some property common to all life. Many have believed that anesthetics directly lower oxygen consumption. But even the consumption of oxygen is not a sufficiently widespread process for there are some organisms which do not require any oxygen and these can be anesthetized.[2] Recently several prominent workers have urged the view that anesthetics have an effect on the permeability of the plasma membrane. It is claimed that they decrease permeability or at least prevent the increase of permeability which is supposed to follow stimulation. R. S. Lillie[3] has summarized the literature on anesthesia and in his excellent review he has brought together most of the evidence in favor of the permeability theory. Some of the opposing evidence is cited by Traube.[4] There are numerous other theories of anesthesia.

Many have tried to associate the phenomenon of anesthesia with what is no doubt one of the most fundamental properties of protoplasm, its viscosity. From the very first description of protoplasm, it has been known that the living substance is viscous. Like all other viscous colloidal substances, no doubt protoplasm can undergo sudden marked increases or decreases in viscosity. These changes in viscosity are among the most outstanding characteristics of those chemical substances which enter into the composition of protoplasm. It is to be expected therefore that viscosity changes play a large part in the mechanics of vital processes.

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[1] Contribution from the Zoölogical Laboratory, University of Michigan.

[2] Vészi, J., *Arch. ges. Physiol.*, 1918, CLXX., 313.

[3] Lillie, R. S., Biol. Bull., 1916, XXX., 311.

[4] Traube, J., *Arch. ges. Physiol.*, 1919, CLXXVI., 70.

In colloidal solutions viscosity changes may be of two sorts. Oftentimes the change is only slight but in other cases there is so marked an increase in viscosity that the colloidal solution loses many of the properties of a liquid and is apparently a solid. Of course it is not truly a solid, for the solid state is properly associated with crystal form. Highly viscous colloidal liquids are known as semi-solids or gels. There is no sharp boundary line between such gels and ordinary liquid colloidal solutions or sols. Every intergradation exists. Colloidal solutions when treated with reagents sometimes undergo gel formation but in other cases there is a precipitation. In the latter instance there is often a transitory increase in the viscosity of the liquid, accompanied perhaps by the formation of an unstable gel, then as the colloidal substance passes out of suspension, the viscosity of the liquid decreases again. In a test-tube there is apparently a sharp difference between gelation and precipitation, but this would not be true within a cell. The entire cell is often smaller than a single flake of the precipitate.

From the first the importance of protoplasmic viscosity changes has been clearly recognized by biologists. Many theories of diverse vital processes have been based on the idea of a change in viscosity or a change in state of aggregation. Claude Bernard himself believed that anesthesia was due to a "semi-coagulation" of the protoplasm.[1] Since Claude Bernard many have held a similar view.

In order to support this view, some authors have endeavored to show a physical effect of anesthetics on various colloidal solutions. Höber and Gordon[2] showed that chloroform vapor and ether vapor tend to prevent the precipitation of a 0.4 per cent. lecithin suspension by barium or calcium chloride. Moore and Roaf[1] found that various anesthetics in high concentration caused a precipitation of the proteins of blood serum. Koch and McLean[2] maintained that anesthetics have no consistent effect on the state of aggregation of lecithin suspensions. Warburg and Wiesel[3] studied series of urethanes, alcohols, nitriles and lactones. They found in every case that the higher members of the series had a more pronounced precipitating action on yeast extracts. The precipitating power was correlated with the power to check fermentation. Battelli and Stern[4] state that all anesthetics possess the power of precipitating nucleoproteins from their watery extracts. Schryver[5] found that the time of gel formation of sodium cholate was lengthened by the presence of anesthetics. Thomas[6] found that anesthetics caused well-marked changes in the viscosity of lecithin suspensions. Generally in higher concentration an increase in viscosity occurred.

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[1] Bernard, C., Leçons sur les anesthésiques et sur l'asphyxie, Paris, 1875.

[3] Höber, R., and Gordon, Dora, *Beitr. Chem. Physiol. u. Path.*, 1904, V., 432.

With the exception of Koch and McLean all of the above authors believe that the physical effects produced by anesthetics upon colloidal solutions are of importance to the theory of anesthesia. But whereas some hold that the effect primarily concerns the viscosity of the substances in the plasma membrane, others apparently believe that the colloids of the cell interior are involved. Not only is there disagreement as to where the effect is produced but there is a decided difference of opinion as to what sort of a change occurs. Some find that anesthetics cause a decrease in viscosity, others claim the reverse. This divergence in results is in part explained by the fact that different authors have used widely different concentrations of anesthetics. Moreover the experiments have been performed upon various types of colloidal solutions.

Although the results on inanimate substances are interesting, it is obviously more significant to measure the viscosity of the living protoplasm itself. This can very simply be done by the centrifuge method. In a recent paper on cell division[1] I have recorded a number of measurements of the viscosity of the anesthetized protoplasm of sea-urchin eggs. The method of measurement is described there as well as in a previous paper.[2] These results give the effect on protoplasmic viscosity of various anesthetics at the very concentration at which they are effective in producing anesthesia.

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[1] Moore, B., and Roaf, H. E., *Proc. Roy. Soc.*, 1906, B. LXXVII., 86.

[2] Koch, W., and McLean, F. C., *Journ. Pharm. Exp. Therap.*, 1910, II., 249.

[3] Warburg, O., and Wiesel, R., *Arch. ges. Physiol.*, 1912, CXLIV., 465.

[4] Battelli, F., and Stern, L., *Biochem. Zeit.*, 1913, LII., 226.

[5] Schryver, S. B., *Proc. Roy. Soc.*, 1916, B. LXXXIX., 176.

[6] Thomas, A., *J. Biol. Chem.*, 1915, XXIII., 359.

One fault that might perhaps be found with some of the prevailing theories of anesthesia is that they are based for the most part on very general evidence. It is only rarely that any one process has been thoroughly studied. And yet in order to understand anesthesia in general it is perhaps essential first to concentrate attention on some one process, preferably a simple one. An explanation of this single case can then perhaps be applied to other cases.

I have studied particularly the process of cell division in sea-urchin eggs. Anesthesia in sea-urchin eggs was first studied by Fühner[3] who interpreted his results as favoring the Overton-Meyer theory. R. S. Lillie[4] later determined the effective concentration for many anesthetics. He also attempted to show that anesthetics decrease the permeability of the plasma-membrane.[5]

In the process of cell division in the sea-urchin egg I have shown that there are certain marked viscosity changes.[6] These are always present. First there is a sharp increase in viscosity and this is followed by a decrease.

What then is the effect of anesthetics on the cytoplasmic viscosity of these eggs? In seeking to answer this question we must study every substance which prevents cell division without killing the egg. Even those substances which produce injurious effects must be considered. According to some writers these are not true anesthetics, but such a distinction is not a wise one. Practically every anesthetic produces some injury if the exposure is

[1] Heilbrunn, L. V., *J. Exp. Zoöl.*, 1920, XXX., 211.

[2] Heilbrunn, L. V., Biol. Bull., 1915, XXIX., 149.

[3] Fühner, H. —., *Arch. Exp. Path. u. Pharmacol.*, 1904, LII., 69.

[4] Lillie, R. S., *J. Biol. Chem.*, 1914, XVII., 121.

[5] Lillie, R. S., *Amer. J. Physiol.*, 1918, XLV., 406.

[6] Heilbrunn, L. V., *J. Exp. Zoöl.*, 1920, XXX., 211.

long enough. After a few hours ether is decidedly harmful to higher animals, and yet no one would question its claim to being an anesthetic. Other anesthetics produce more marked injuries. There is every gradation between those which produce slight injury and those which cause considerable damage. Where then shall we draw the line? I propose to consider as an anesthetic any substance which stops a vital process without killing the cells in which the process occurred. We can then distinguish between the less injurious and the more injurious anesthetics.

In the following table all the substances listed produce anesthesia in the sea-urchin egg. The concentrations represent per cents. in sea-water. At the concentration indicated in the first column, in every case a marked lowering of the viscosity of the egg protoplasm could be demonstrated. This was proven by centrifuge tests. Normal eggs in sea-water and anesthetized eggs were centrifuged simultaneously, the normal eggs being placed in one tube, the anesthetized eggs in another. At a speed which produced no movement of granules in the cytoplasm of the normal eggs, the granules in the cytoplasm of the anesthetized eggs were thrown completely into one half of the egg. The difference was in every case very striking. The normal eggs appeared evenly opaque. The anesthetized eggs had half or more of their cytoplasm perfectly transparent. The viscosity of the anesthetized cytoplasm was undoubtedly many times less than that of the normal eggs.

	Anesthetic concentration	Lethal concentration
Ether	2 %	4 %
Chloroform	0.13%	1 % (emulsion)
Chloral hydrate	0.25%	1 %
Nitromethane	2 %	3 %
Paraldehyde	4 %	8 %
Acetone	5 %	10 %
Ethyl nitrate	0.4 %	
Ethyl acetate	2 %	5 %
Ethyl butyrate	0.25%	0.5%
Acetonitrile	4 %	5 %
Propyl alcohol (normal)	1 %	
Amyl alcohol	0.6 %	1 %
Phenyl urethane	4/5 saturated	saturated (= < 0.5%)
Ethyl urethane	1.5 %	3 %

The concentration of anesthetic which produces a marked lowering of protoplasmic viscosity is the exact concentration which prevents cell division. Lower concentrations produce less decided effects on cytoplasmic viscosity and in these cell division is more apt to occur. As the concentration of the anesthetic is increased, the decrease in cytoplasmic viscosity becomes more and more pronounced until suddenly a turning point is reached. At this point gelation or coagulation occurs. Eggs subjected to such high concentrations of anesthetic undergo a decided increase in cytoplasmic viscosity. When these eggs are centrifuged at high speed the cytoplasmic granules remain fixed and do not move. Compared with normal eggs the protoplasm of these coagulated eggs is much more viscous. The concentrations noted in the second column of the table are those which were found to produce a sharp increase in protoplasmic viscosity. Such an increase in viscosity is in general irreversible. In practically every case the eggs did not recover.

My results with ether agree fairly well with the older observations of Heilbronn[1] on plant protoplasm. Heilbronn's measurements were made by timing the drop of starch granules. He found that dilute ether solutions caused a decrease in protoplasmic viscosity; more concentrated solutions caused increased viscosity. Heilbronn was inclined to associate anesthesia with the more concentrated solutions, which caused a stiffening in the protoplasm, a "Plasmastarre." Perhaps this is true for plant cells, but it is rather difficult to decide when plant cells are anesthetized. Heilbronn found that the "Plasmastarre" was in general reversible.

In medicine cold is a well-known anesthetic. Low temperatures also anesthetize sea-urchin eggs. With decreasing temperatures the protoplasm becomes less and less viscous. Even at 10° C. there is a noticeable difference as compared to room temperature. At — 3° C. centrifuge tests both with fertilized and unfertilized eggs showed a decided lowering of viscosity. In such eggs cell division is prevented, although the eggs remain uninjured. Temperatures somewhat lower than — 3° produce an increase rather than a decrease in viscosity. If the sea-water in which the eggs are contained is allowed to freeze, the egg cytoplasm becomes coagulated. Such eggs are permanently injured by the treatment. In one experiment eggs were exposed to a temperature of —6° C. for 10 minutes. In spite of the fact that no ice was observed in the tube containing the eggs, the protoplasm when tested was found to be coagulated. Thus in the case of cold also there is apparently a turning point, at which the viscosity of the cytoplasm no longer decreases but suddenly shows a marked increase.

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[1] Heilbronn, A., *Jahrb. wiss. Bot.*, 1914, LIV., 357.

When sea-water is diluted, the resultant hypotonic solution is one of the best (*i.e.,* least injurious) anesthetics. A solution made up of equal parts of sea-water and distilled water is an excellent anesthetic. There is nothing unusual about this, for is has long been known that water anesthetizes nerves. This knowledge dates back as far as 1869,[1] and water has occasionally been used in various surgical operations. In the sea-urchin egg those dilutions of sea-water which prevent cell division are the very ones which produce a marked decrease in cytoplasmic viscosity. On the other hand, too great a dilution causes coagulation; this effect is produced when the eggs are dropped into distilled water.[2]

Not all anesthetics cause a decrease in cytoplasmic viscosity. Some produce quite the opposite effect.

Many students of anesthesia confine their attention to fat solvent anesthetics. This in spite of the fact that magnesium sulphate in concentrated solution is apparently the best anesthetic for most marine invertebrates. As far as I can discover magnesium sulphate was first used by Tullberg.[3] Its effect appears to be largely an osmotic one, for the salt acts best in hypertonic solution. This is also indicated by the observations of Loeb[4] on hydroids. Loeb found that the regeneration of hydroids could

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[1] Braun, H., Local Anesthesia, English translation by Shields, Philadelphia. 1914, p. 62.

[2] Heilbrunn, L. V., BIOL. BULL., 1915, XXIX., 149.

[3] Tullberg, T., *Verhandl. d. biolog. Vereins Stockholm,* 1891, IV., Nr. 1–2, p. 4, cited after Gerould, J. H., *Bull. Muss. Comp. Zoöl.,* Harvard, 1896, XXIX., 121.

[3] Loeb, J., Untersuchungen zur physiologischen Morphologie der Thiere, Würzburg, 1891–1892.

be reversibly prevented by placing them in sea-water concentrated by evaporation. This concentrated sea-water acted as an anesthetic. Loeb[1] also found that hypertonic solutions made by adding sodium chloride to sea-water prevented segmentation in the sea-urchin egg. The eggs were able to resume division on being transferred to normal sea-water, although the division process was somewhat altered.

I showed in 1920 as well as in 1915[2] that hypertonic solutions greatly increase the viscosity of sea-urchin egg cytoplasm. Hypertonic solutions are not the only anesthetics that act in this way. Dilute solutions of potassium cyanide have long been known as anesthetics for sea-urchin eggs. In anesthetic concentration, I have shown that cyanide solutions increase protoplasmic viscosity and render irreversible the normal gelation which occurs in the course of the mitotic process. Chloretone solutions act in a similar way.

Thus *there are two types af anesthesia in the sea-urchin egg.* In the one the viscosity of the cytoplasm is decreased, in the other it is increased. The two types of anesthesia differ also in the course of their action on the egg. When the viscosity is decreased sufficiently, the mitotic spindle is always prevented from forming. Even after the spindle has appeared exposure to ether or to cold causes the astral rays to disappear. On the other hand, anesthetics like hypertonic solutions and cyanide do not have such an effect. As is well known many hypertonic solutions produce asters and spindles. In dilute solutions of cyanide the mitotic process continues until the spindle is formed and then stops.

Of course in a way both types of anesthesia have one common effect upon the protoplasm. In both cases the protoplasmic viscosity is held fixed. Either the protoplasm is kept in a fluid state, or it is made to stay in a stiff condition. The end result is the same. The viscosity changes upon which mitosis depends can not occur, and the egg is anesthetized.

It is believed that not only in mitosis but in other processes as well, two types of anesthesia occur. This is borne out by a series of experiments now in progress with *Hydra*. Every student of zoölogy has watched this little animal contract on mechanical stimulation. I have been able to show that when *Hydra* is anesthetized, so that it no longer responds to mechanical stimulation, two types of anesthesia may be distinguished. In the one type, the *Hydra* tends to contract its tentacles and to remain contracted. This type of anesthesia is produced by ether and cold and in general by those substances which lower the viscosity of sea-urchin egg protoplasm. On the other hand, those substances which increase protoplasmic viscosity tend to anesthetize the *Hydra* in an expanded state.

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[1] Loeb, J., *J. Morph.*, 1892, VII., 253.

[2] Loc. cit.

STUDIES ON INSECT SPERMATOGENESIS.

I. The History of the Cytoplasmic Components of the Sperm in Hemiptera.

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Introduction.

No one who has noted the trend of cytological study during the last twenty years can fail to be impressed with the contrast between the progress that has been made in the analysis of the nucleus, and more particularly of the chromosomes, and the relatively doubtful outcome of parallel investigations on the cytoplasm and its formed elements. Perhaps this is because no great, illuminating hypotheses have succeeded in doing for the cytoplasmic structures what the theories of heredity and sex have accomplished for the chromosomes. At all events, it is evident that we are still on very uncertain ground in regard to many questions connected with the cytoplasmic constituents, especially such as relate to the physiology and reproduction of the cell. It is the purpose of this paper to deal with certain of these problems presented by the formed elements of the cytoplasm, more particularly the mitochondria and the Golgi apparatus.

In the case of the cytoplasmic structures, as in that of the chromosomes, the maturation period of the germ cells and the subsequent phenomena of fertilization offer the best setting for a study of many problems of the cell. Especially is this true of spermatogenesis, where the growth period of the spermatocytes and their subsequent division and differentiation into the sperms afford material for a critical study of a variety of cellular structures. The activities of the various elements of the germ cells take on an added interest when one considers that the result is to be the formation of sperm which must carry the total hereditary complex of the male, locked up with a mechanism for active locomotion and for initiating the cleavage of the egg. It follows, therefore, that if we are rightly to estimate the rôle of the sperm in fertilization we must first gain a correct conception of the processes leading to its formation. What structures are present in the germ cells? How are they distributed to the spermatids? What is the rôle of each in the differentiation of the spermatozoön? It is from the standpoint offered by such questions that these studies have been undertaken. It was originally intended to publish the results in one complete paper, but the delays incident to printing at the present time have made it seem preferable to publish them now in briefer form, to be followed later by a more detailed account. This paper contains, therefore, a summary only of the more important points, especially such as bear more immediately on certain general cell-problems.

For this study the insects offer many obvious advantages, not the least of them the fact that the history of the nucleus has been so thoroughly studied in this group that a trustworthy outline is already at hand upon which to work out further details. I have accordingly drawn the material for this study from Hemiptera belonging to the Family *Pentatomidæ,* of which the so-called "stink-bugs" are common examples. Of many forms examined, I have made use particularly of *Murgantia histrionica* Hahn, and *Euschistus euschistoides* (=*fissilis*) Voll. Other species of *Euschistus* and several other genera (*Arvelius, Brochymena* and *Nezara*) have also been used in cases where some particular point was presented with unusual clearness or afforded interesting comparisons. Among these various genera minor differences occur, but in general the phenomena are essentially the same, the factor deciding the use of one or another genus for a given purpose being usually the size of the cells.

Much of the work published heretofore on the formation of the sperm has suffered, obviously, from an insufficient technical treatment. The extensive use of acetic acid in the fixative has been the chief source of failure, as Gatenby has recently insisted, for it has turned out that certain important cytoplasmic structrues—mitochondria and Golgi apparatus—are both of lipoid nature and hence easily destroyed by fat-solvents. I have tried to obviate this difficulty by recourse to a very extensive variety of fixing and staining reagents, a review of which will be given hereafter. For the present purpose, a few general notes will suffice.

For the purely topographic study of sperm formation, and for working out the finer details connected with the transformation of the nucleus into the sperm head, strong Flemming followed by Fe-hematoxylin and any desired counter stain, is still the most satisfactory technique. For the mitochondria I have tried a number of methods, but by far the best for most purposes is Benda's well-known alizarin-crystal violet stain for which *Euschistus* has proven rather favorable material. For the Golgi apparatus I have tried all the better known methods, including those of Cajal, Kopsch, and Gatenby, with all of which results more or less satisfactory were obtained. None of them, however, proved of any value in studying spermatogonia and early spermatocyte stages, and they were almost equaly defective for the seprmatocyte divisions. Fortunately, in the course of my experiments I found a modification of the Kopsch method which proved to be very selective in its action and made possible a complete study of the Golgi apparatus in the earliest stages, and also throughout the maturation divisions. A description of this method has already been published (Bowen, '19), to which reference may be made for the details. In subsequent statements, I will refer to this method as "modified Kopsch."

I would like to take this opportunity of acknowledging my great indebtedness to Professor E. B. Wilson, who has placed at my disposal his many preparations of Hemipteran germ cells, and whose suggestions and kindly criticism have been of invaluable assistance to me in the preparation of this paper. I am indebted also to Dr. Franz Schrader and Mr. S. C. Dellinger for assistance in the collection of material; to Mr. H. G. Barber who has identified much of my material; and to Miss Helen Daniels who has made careful copies of my original figures for the purpose of this publication.

Observations.

I. *Structure of the Testis and Stages of Spermatogenesis.*

The progress of spermatogenesis may be followed most easily by dividing the process as a whole into a series of more or less arbitrary steps or stages, based for the most part upon the condition of the chromatin in the nucleus. In the case of the Hemiptera the nuclear changes have been studied with exceptional thoroughness particularly by Wilson and Montgomery, and the steps from spermatogonium up to a late period in sperm formation are completely determined. I will, therefore, make use of the descriptive terms employed by Wilson ('12) in his outline of the stages, since they furnish well known landmarks and moreover designate periods of profound nuclear changes which, it is interesting to note, are accompanied by simultaneous changes in the cytoplasm.

(*a*) *Polymegalous Spermatocytes and Sperms in the Family Pentatomidæ.*

Hemiptera of the Family *Pentatomidæ* possess two compact testes, each enclosed in a delicate sheath of connective tissue, which is continued into the body of the gland in the form of septa or partitions dividing it into a number of compartments or lobes. The latter are arranged parallel to the long axis of the testis and vary in number (in the forms which I have examined) from three to seven, the number being constant, however, for any particular species. Speaking generally, the plan of the testis is not unlike that of some other families of Hemiptera, but in its details there is one notable difference in respect to which this particular family is unique, so far as known. I refer to the curious fact, to which Montgomery ('98) first called attention, that in *Euschistus* certain lobes of the testis are composed characteristically of spermatocytes unusually large or unusually small ("dimegaly") as contrasted to those of other lobes, which latter for want of a better term might be called "normal" in size. Subsequently Montgomery ('10) published a much more complete account of this unusual condition, establishing the fact that in *Euschistus* sp., two of the lobes consist of unusually large spermatocytes, a single lobe of unusually small ones, while the remaining three lobes are normal. He found the spermatogonia and early spermatocytes to be all of the same size, but that following the synaptic period the size differences are established by differential growth. The relative differences he found to be always the same in any given lobe, all the spermatocytes in a lobe being equally affected. There are accordingly three distinct sizes of spermatocytes which give rise to three proportionally different spermatids and these in turn to three kinds of sperms with clearly marked morphological differences. But in the whole series the amount of chromatin seemed to be identical, the chromosome sets of the maturation divisions being similar in every respect. Montgomery examined a number of related genera but found no such size differences, or at least no conspicuous ones.

My attention was first attracted to this problem by the discovery that *Murgantia histrionica* also exhibits size-differences similar to those of *Euschistus* but much less conspicuous, two lobes having large and three lobes normal-sized spermatocytes. The expected two kinds of spermatids and sperms (Figs. 34 and 35) were also found. These facts aroused the suspicion that "dimegalous sperm" were by no means unique to *Euschistus* as Montgomery had supposed, and accordingly it occurred to me to make a survey of the whole Family *Pentatomidæ* with respect to this particular point. The results fully confirmed my suspicion, for of thirty-seven species examined, twenty showed visible size differences, sometimes of three classes, sometimes of two. Furthermore, every degree of relative difference was found, ranging from the inconspicuous difference in *Murgantia* up to a most remarkable difference in *Arvelius,* where the whole testis is dominated by the large generations of spermatocytes. I found, further, that every part of the cell shares in this differential growth except the chromosomes—the nucleus, cytoplasm, plasmosome, mitochondria, Golgi apparatus, acrosome, centrioles, spindle and chromatoid body being of a size roughly proportionate to that of the cell as a whole. It might be supposed that the morphology of the cytoplasmic constituents would be affected by these size differences, and indeed some differences do occur, but they appear to be of quite secondary importance. Finally, I have found that the processes by which the sperm head is differentiated differ rather strikingly in the large and small cell generations, as will be indicated.

Concerning the significance of these facts, we are at present quite in the dark, nor do we know that the "polymegalous" sperms are of equal value in fertilization. I have nevertheless found these size differences of great practical value in cytological studies, for one has at his command on the one hand a complete series of stages in miniature, and on the other a selection of the same stages reproduced on a larger scale. In the description which follows I shall refer to the dimegalous cells simply as "large" and "small," since the unusually small generation presents no points of special interest.

II. *Stages Preparatory to Sperm Formation.*

The history of the nucleus from the spermatogonial cell through the close of the second maturation division has been so minutely studied in the Hemiptera (see more particularly Montgomery ('11) and Wilson ('12)) that further description would be superfluous. I will accordingly proceed at once to a consideration of the formed elements of the cytoplasm with which I have been more especially interested.

A brief reference must first be made to the centrioles, since a knowledge of their behavior is essential to a proper understanding of other cytoplasmic phenomena. At the close of the final spermatogonial division the cells are so small and crowded that the centrioles can not be identified. They may first be made out with certainty in the spermatocytes during the growth period and, when once relocated, can easily be followed through to the close of the second maturation division. The point to be noted is that when the centrioles reappear they are already located on opposite sides of the nucleus in position for the formation of the first maturation-spindle. Furthermore, the nuclear membrane bulges out and the cell wall is drawn in opposite each centriole (Fig. 11), so that the centrioles touch (or nearly touch) the wall of both nucleus and cell, the cytoplasm in the neighborhood of the centrioles being thus greatly restricted. A section through both centers accordingly gives a very characteristic picture (see Montgomery ('11), Fig. 63).

Of the remaining cytoplasmic constituents, the mitochondria are the best known and have already been described in *Euschistus* by Montgomery ('11). His account was, however, based on faulty material and I have found it necessary to make a reexamination of the whole subject. The following abridged account has been drawn from *Euschistus euschistoides.*

The earliest spermatogonia which I have studied are from the cysts of large pyramidal cells arranged in a rosette, (see Montgomery ('11), Fig. 3), and in these cells the mitochondria are aggregated into a dense mass fitted closely to the nuclear membrane like a cap. The position of the latter with reference to the long axis of the cell is variable, and thus no constant polarity is visible. From this point through to the diplotene stage the mitochondria are always thus massed together (Fig. 1) except during the spermatogonial divisions when they spread throughout the cytoplasm in what appears to be a tangle of exceedingly delicate threads and granules. Montgomery supposed the mitochondrial cap to be an idiosome (idiozome), and he concluded naturally that there were no mitochondria in the spermatogonia—or at least that they must be chemically different from those of later stages. In this he was clearly misled by inadequate technique. In point of fact, beginning at least with the rosette cells the mitochondria may be traced through to the mature sperm without a break in continuity or any fundamental changes in staining reaction.

With the diplotene the spermatocytes begin to increase markedly in size, and the cap of mitochondria likewise undergoes a corresponding growth (Fig. 3). At the same time it grows looser in texture and in the large cells is seen actually to consist of a tangle of fine threads. As the growth period is entered upon, the threads begin to migrate out into the general cytoplasm, and in a short time become distributed uniformly throughout the cell (Fig. 5). This movement is accompanied by an increase in the diameter of the threads and probably in their length, but their actual number seems to be little if at all affected. In the small cell generations the breaking up of the cap is somewhat different in detail, but the final result in all the cells is the same—the mitochondria are distributed throughout the cytoplasm as a mass of threads in what appears to be a hopeless tangle. This condition continues throughout the growth period—except in the large cells, where the threads may undergo more or less extensive granulation—the mass of threads enlarging with the general growth of the cell as a whole.

With the prophases is inaugurated a most interesting series of events to which I would like to call special attention. In this particular period, including the prophases of the maturation divisions and also the divisions themselves, the essential phenomena are the same in both large and small generations; but the cell pictures differ somewhat in the fact that the mitochondria in the small cells are in general thread-like, while in the large cells they are in the form of granules and rather thick rods of no great length. The following account is based on the small cells, brief details of the large ones being added by way of comparison.

The early prophases of the first maturation division present nothing of special interest. Montgomery's figures of the mitochondria in this stage are essentially correct, except that the threads appeared to him very much thinner than is the case when the Benda stain is employed. In the middle prophases a change in the arrangement of the threads becomes obvious though its exact nature is difficult to state. Occasionally in a favorable cell it is possible to make out a number of ends of threads directed toward a centriole, but at this time one would certainly never guess the nature of the ultimate arrangement of the threads of which these uncertain phases give the first inkling. In the final prophases the rearrangement of the threads is completed and the nature of the whole process becomes entirely clear. As now appears, the tangle in which the threads seemed to be at the close of the growth period was much more apparent than real. The result of the rearrangement of the threads is best seen in sections containing one or both of the centrioles which are now located at opposite sides of the nucleus, thus marking out the long axis of the future spindle. Hence it is convenient to anticipate the spindle and speak of polar and lateral aspects of the spermatocyte, in the sense in which those terms are customarily applied to metaphase figures of a dividing cell. In a lateral view of the late prophase (Fig. 11), the arrangement of the mitochondria is very characteristic. In the cytoplasmic zone immediately surrounding each centriole the mitochondria are entirely absent, the limit of their distribution being abrupt and constant. Thus the mitochondria come to form a broad, equatorial girdle enclosing the nucleus, and, as subsequent developments show, the spindle, while the centrioles occupy the open ends of the sheath-like girdle. Furthermore, the zone of cytoplasm around the centrioles is delimited by the ends of threads, of which a few can be seen in lateral view—the main bulk of the threads being lost in an equatorial tangle impossible of analysis. The actual arrangement in the vicinity of the centrioles is rendered much clearer by a study of polar views or of sections cut obliquely through one centriole. In direct polar views (Fig. 12), especially if the cell is somewhat flattened, the paired centrioles are seen to occupy the center of a cytoplasmic area which in preparations properly fixed is seen to be the area toward which the astral rays are converging. Around the edges of this clear area are arranged the ends of the mitochondrial threads which radiate out toward the equator of the cell like so many meridians of longitude. In oblique sections through one polar area (Fig. 13), the polarization of the threads toward the centrioles is very clear, and in such views one can generally follow the individual threads for some distance. It appears that the threads are quite variable in length, some being relatively short while others are long, but generally speaking any one thread seems to be oriented toward a centriole at one end only, while the free end becomes more or less bent and twisted and is lost in the tangle which encircles the equator of the cell. In cross sections through the subequatorial region, one sees for the most part only the ends of threads. It is clear from the foregoing that the maze of threads in the spermatocytes is untangled during the prophases, apparently under the influence of the centrioles, resulting finally in the polarization of most (all?) of the threads toward the opposite centrioles, while in the equatorial zone the free ends of the threads apparently become lost in an inextricable tangle.

Meanwhile the nuclear membrane fades out, the chromosomes take up their position on the spindle and the metaphase figure of the first maturation division is complete (Fig. 14). In sections through the long axis of the spindle numerous threads can be made out directed toward the centrioles, but in polar view the orientation of the threads is usually less clear than in the prophases, a condition which seems to be connected purely with the spatial relations inside the cell. Thus by reference to Fig. 11 it will be seen that in the prophases the nucleus and the cell wall are closely approximated adjacent to the centriole, so that the ends of the threads are automatically located in the same optical plane. When the nuclear membrane breaks down, however, the spindle region is relatively small in comparison, and the mitochondria tend to spread out in the extra space, though they never encroach on the spindle area proper. So it happens that the striking effect of polarization is somewhat obscured, but it is perfectly clear that the orientation remains fundamentally unaltered.

The chromosomes now divide and the daughter plates begin to draw apart (Fig. 16), while the cell itself elongates very considerably, so that the distance between the centrioles is increased. The result of these movements upon the mitochondria is twofold. In the first place the polarized ends of the threads are straightened out along the spindle and in the second place they are drawn along with the diverging centrioles. As an obvious result the opposite ends of the threads are passively drawn out of the equatorial tangle, and in an early anaphase (Fig. 16) the threads, now arranged in lines more or less parallel to the spindle, come to form a sort of "palisade," or sheath, encircling the mitotic figure. The constriction of the cell wall meanwhile develops rapidly and soon comes in contact with the mitochondrial palisade. The threads, however, show no tendency to divide autonomously or to be divided mechanically, and as the constriction deepens they are carried inward by the advancing furrow. Thus the palisade as a whole is constricted very markedly in the equatorial plane, while the opposite ends flare widely, leaving an open space in which lie the daughter chromosome groups. Finally, the constriction reaches the spindle, which, by its tendency to persist as a connection between the daughter secondary spermatocytes, appears to be of a rather firm consistency. The mitochondrial threads which happen to lie immediately in the path of the constriction are thus at last caught between the spindle and the cell wall and seem to be severed mechanically into two parts (Fig. 17). That the threads are actually cut in two, not merely withdrawn into the daughter cells, is made practically certain by the whole series of anaphase phenomena, and this interpretation is strengthened by a study of late anaphases in which a few threads still cross from one cell to the other while the majority are already severed (Fig. 17).

The arrangement of the threads with reference to the spindle is soon obscured and they spread out in each second spermatocyte in preparation for the next maturation division which occurs immediately. As many cytologists have described (see the accounts of Wilson ('12) and Montgomery ('11)), during the anaphases of the first maturation division the halves of each centriole separate in a direction at right angles to the old spindle, so that the main axis of the second maturation spindle is at right angles to that of the first. The centrioles are indeed already in position at the conclusion of the anaphase, so that the distribution of the threads in the daughter cells can be followed with reference to a possible further influence by the centers. As a matter of fact, the threads are rearranged around the centrioles as before, and the second maturation division is in general a half-size replica of the first, at least up to the cutting in two of the "palisade." The formation of the nebenkern from the mitochondria thus distributed to the spermatid will be taken up in the next section.

In the large cell generations the facts are essentially the same, but the mitochondria are comparatively short, heavy rods with numerous granules intermixed. These rods are oriented around the centrioles in a most striking manner. Their behavior at the close of the first maturation division is of special interest, for at this time the influence of the centrioles on the rods is very clearly demonstrated. In fact, as the "palisade" breaks up the rods can be seen diverging toward the centrioles in two groups, showing with the greatest clearness that the movements of the mitochondria during the entire division period are very definitely oriented with respect to the centrioles.

To summarize the behavior of the mitochondria in preparation for the formation of the sperm, it may therefore be said: (1) that in the early spermatocytes the mitochondria form a dense, nuclear "cap," (2) which is broken up during the growth period and distributed in a tangle of threads throughout the cell; (3)

that in preparation for and during the maturation divisions the threads are under the visible "control" of the centrioles, (4) and that by this means each spermatid receives an accurate quarter of the mitochondria contained in the first spermatocyte.

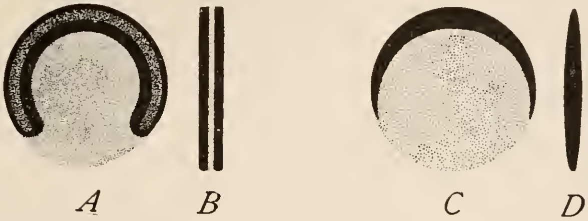
One important cytoplasmic structure remains to be considered, viz., the *Golgi apparatus,* a cell element whose rôle in spermatogenesis has thus far been largely mistaken or overlooked, but which, as I hope to show, must play a significant part in all future analysis of the sperm. The term "apparatus," in its original meaning, is rather a misnomer in the Hemiptera, for in these forms this structure is generally composed of many separate *Golgi bodies* (Fig. 7). To one long accustomed to the appearance of Flemming preparations of insect germ cells, the Golgi preparations are a revelation; for the mitochondria and chromosomes fade into a shadowy background upon which the Golgi bodies appear in brilliant black. Indeed a successful preparation is so nearly diagrammatic that the main outlines can be made out almost at a glance. For the purposes of this report, I have abridged the description of the Golgi bodies, attention being centered principally on their behavior in the maturation divisions. This account also is based on *Euschistus.*

For a starting point we may, as in the case of the mitochondria, take the spermatogonia in the "rosette" stage. In these early generations of spermatogonia the Golgi apparatus consists of a few scattered bodies which show no particular orientation with respect to any other part of the cell. In later generations the number seems to be slightly increased and a tendency for the bodies to collect in the neighborhood of the mitochondrial "cap" is apparent. Finally in the last spermatogonial generation (or possibly stage *a* of Wilson), this orientation is completed (Fig. 1), and the Golgi bodies are definitely restricted to that portion of the cell in which the mitochondria are aggregated. In dividing spermatogonia the Golgi bodies are broken up into small granules which seem to have no definite method of distribution to the daughter cells.

The synaptic stages offer nothing of interest but, as the mitochondria spread out in the diplotene, the Golgi apparatus likewise enters upon a period of growth and activity. As in the case of the mitochondria the early steps in this process are much clearer in the large cells, but in most respects the small cells are the same, and important differences will be pointed out by way of comparison. In the large cells the individual Golgi bodies spread out more or less as do the mitochondria and at the same time begin to increase in size (Fig. 4). Growth is rapid and is continued up to the end of the growth-period proper. The Golgi bodies soon begin to migrate out into the general cytoplasm, their movement seeming to be approximately synchronous with that of the mitochondria. Thus they soon come to be scattered in hap-hazard fashion throughout the cytoplasm (Fig. 6), a condition which is maintained throughout the growth-period. Their number seems to be about twenty-five or thirty but it is not possible to make a very accurate count because each cell extends over several sections and the bodies may vary as to impregnation. The point particularly to be emphasized is that the Golgi bodies never at any time form a condensed aggregate in any way comparable to the so-called "Nebenkern" of pulmonates or the idiosome or "sphere" of many other forms—particularly vertebrates.

In the small cells a similar growth and migration of the Golgi bodies occurs, but it is characteristic of these generations that the general distribution is preceded by a stage in which the Golgi bodies become more or less completely condensed into one or several masses. Fig. 2 shows a cell in the early growth period in which most of the Golgi elements have condensed into a single body reminiscent of the idiosome of the opossum as figured by Duesberg ('20). It should be noticed that even in the case figured, a few Golgi bodies have failed of incorporation in the "idiosome" and usually the fusion is much less complete, resulting in several masses with numerous outlying, separate bodies. The condition in the large cells (where this phenomenon never occurs), together with the appearances often found in material fixed with Flemming and the customary failure to form a close aggregate even in Golgi preparations, leads me to believe that the fusion may possibly be in part a result of poor fixation. However, the constancy of this phenomenon argues something in favor of its reality, and the similarity to the idiosome and "Nebenkern" is very suggestive. It is this body which Montgomery called the "sphere" (see Montgomery ('11), Fig. 48). His figures are characteristic of preparations fixed for instance in Benda's Flemming, in which the shape of the "sphere" seems certainly to be an artifact.

The shape of the Golgi bodies is much the same in all the cells, but their size varies with that of the cell as a whole. In the early growth stages (Fig. 4) the bodies appear as rods, straight or curved, which impregnate intensely, and when they have become large enough to admit of a closer study of their structure it becomes evident that the intensely black portion is accompanied by a substance which impregnates little or not at all (Fig. 6). Usually the rods are bent around this substance enclosing it more or less completely. The two portions taken together form the Golgi bodies, which are *plate-like* and not spherical. Viewed on edge, these plates or discs present a most characteristic appearance, for the rods are actually double and thus the impression is given of a pair of rods lying side by side (Text-figure 1*b*) and separated by a clear space. So constant is this apparent "split" that from very early stages until the final condensation of the Golgi apparatus in the spermatid, it is the most striking characteristic of this kind of technique. In the late growth period in *Euschistus*, the structure of the Golgi bodies is especially clear (Fig. 7). Each disc-like body is clearly composed of two substances, a peripheral portion impregnating very densely and enclosing, sometimes completely, a plate of material which is only slightly blackened. The peripheral portion often appears as a double rim, the meaning of which is not clear (Text-figure 1*a*).



TEXT-FIGURE 1. Schematic representation of a single Golgi body. *A*, after fixation by the "modified Kopsch" method; *B*, the same as seen on edge; *C*, after fixation and staining by chrome-osmium-hematoxylin methods; *D*, the same as seen on edge.

Viewed on edge the bodies exhibit the characteristic "split" (Text-figure 1*b*)—a feature very possibly of wide occurrence as it seems to be referred to in several published accounts of the Golgi apparatus. Following the so-called chrome-osmium methods much used by Gatenby, the appearance is quite different (Text-figure 1*c*). The peripheral rim stains only as a dark crescent on one side of the disc of non-staining substance ("archoplasm" of Gatenby)—or occasionally the rim may stain more completely, approaching the result obtained by impregnation with osmic acid. By these methods I have, however, been unable to demonstrate the "split" in an edgewise view (Text-figure 1*d*). Without going further into the morphology of the Golgi bodies, I would like especially to call attention to their obviously duplex structure as demonstrated by the staining reactions, a feature to which I shall return in a later section.

In the final growth period of the large cells it is not uncommon for the Golgi apparatus to undergo secondary changes. Thus in *Euschistus* the bodies tend to fuse with each other, the number in a single cell being reduced to ten or twelve. In *Brochymena* this fusion is carried much further, and great, amorphous masses are formed, while in *Nezara,* on the other hand, the individual bodies are very small. That these various morphologic differences are of no particular importance is indicated by the fact that in the subsequent divisions the general behavior is the same in all three genera.

The spermatocytes now enter upon the prophases, and until the spermatids are finally formed the behavior of the Golgi bodies is of great interest. The coincident series of events through which the mitochondria and the chromosomes are passing should be borne continually in mind, for only by such a composite picture can one form a proper conception of the mitotic process as a whole. As in case of the mitochondria, it is the small cell generations which offer the best material for the general study of the Golgi bodies during the prophase and division stages, and I will accordingly refer to the large cells only by way of comparison.

The early prophase probably marks the beginning of the steps preparatory to the division of the Golgi apparatus, but it is only in the middle prophases that the results become clearly evident. Then it appears that the Golgi bodies, moving from all parts of the cell, have been gradually forming a narrow belt or girdle encircling the nucleus in what subsequently proves to be the equatorial region. Viewed from one pole, the discs form a more or less complete ring (Fig. 8) and are in general so oriented that their flat surfaces are turned toward the nuclear membrane, the edges with the characteristic "split" being thus directed toward the observer. The middle prophase now passes rapidly into the late prophase and in this stage the ring of Golgi bodies undergoes a most characteristic change. This consists of an autonomous fragmentation of all the Golgi bodies so that in the place of the ring of large discs, there is now a throng of much smaller bodies (Figs. 9 and 10). In some, a composition similar to that of the growth period discs can often be made out, but usually from this point on the morphological distinctions become more or less obscure owing to the small size of the bodies and faults in impregnation. In the small cells each Golgi body appears to be divided into two parts, but I do not wish to emphasize this point. In the large cells each of the Golgi elements fragments into many pieces, and the process of fragmentation can actually be followed. It is certain that the division is always at right angles to the "split," and the latter, therefore, however well adapted it may appear for purposes of division, never serves that end. Further, in the large cells the division clearly includes the central portion of the disc, resembling very strongly the figures given by Gatenby as representing the division of a Golgi body (see Gatenby ('19*b*), Fig. 14). The nuclear membrane now fades away and the metaphase figure is rapidly formed.

Before taking up the actual division stages, a word may be said concerning the nomenclature of the Golgi elements. The scattered condition of the Golgi apparatus throughout the growth period renders the use of the term Golgi "bodies" a most convenient one. Their fragmentation introduces a complication which for practical purposes I propose to avoid by the use of the term *dictyosome,* a word coined by Perroncito (see especially his paper of 1910) to denote the pieces into which the Golgi apparatus was divided during mitosis. If its meaning be restricted in this original sense, the term may prove very useful, especially in such a case as the present one.

To return to the metaphase figure, apparently by the time the spindle is completed the ring of dictyosomes has already begun to spread out, and soon these bodies migrate throughout the space between the centrioles and peripheral to the spindle. At first all semblance of order is lost and the dictyosomes seem to be scattered at random around the spindle. But presently it can be seen that they are migrating toward the poles of the spindle in a very orderly way, and as the equatorial region gradually becomes clear, they are seen to be collecting in approximately equal groups in the vicinity of the centrioles (Fig. 15). In *Euschistus* this grouping is always somewhat loose, especially in the large cells, but in *Brochymena* (Fig. 15) the dictyosomes collect in two very accurate rings encircling the poles of the spindle, and presenting in polar view a most characteristic appearance.

During this entire process of *dictyokinesis*[1] the chromosomes retain their position in the metaphase plate, and only when the two groups of dictyosomes have completed their journey toward the centrioles do the chromosomes resume their activity as the anaphase begins. As the cell draws out, the groups of dictyosomes are also separated, so that for a brief interval the separating chromosome plates lie in a clear intermediate zone. Soon, however, they overtake and pass through the ring-like groups of dictyosomes and, as the daughter chromosomes complete their movement to the spindle poles, the groups of dictyosomes break up and begin to wander out through the general cytoplasm of the cell, becoming scattered apparently in all directions. During the period just prior to, and following the final anaphase the dictyosomes seem to undergo a second fragmentation, so that those of the second spermatocytes are markedly smaller in general than were those of the first. The dictyosomes form a belt, rather less definite in contour, around the chromosome plate of the second maturation division, and the process of dictyokinesis is repeated. Indeed, the steps are so similar to those of the first maturation division that without some familiarity with the particular genus one happens to be observing it is impossible to say off-hand whether the division is that of a first or second spermatocyte. The fate of the dictyosomes in the telophase of the second maturation division will be taken up in connection with the formation of the sperm.

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[1] A term employed by Perroncito to denote the division phenomena of the Golgi apparatus. Compare the parallel term, *karyokinesis*. See especially, Perroncito ('10).

To summarize the behavior of the Golgi apparatus in these stages: (1) in the early spermatocytes the Golgi apparatus consists of a number of scattered bodies which in later spermatogonial generations are collected around (and in?) the mitochondrial cap; (2) during the growth-period these bodies spread throughout the cell, and take part in its general growth; (3) they are finally accurately oriented with respect to the first maturation-spindle apparently through the influence of the centrioles; (4) then, having undergone autonomous fragmentation, the resulting dictyosomes are actively distributed during the maturation division under the visible control of the centrioles, (5) by which means each spermatid receives an accurate quarter of the Golgi apparatus present in the first spermatocyte.

III. *The Formation of the Sperm.*

So intricate and extended are the processes by which the mature sperm is differentiated from a spermatid, that at this time I can scarcely do more than indicate the main features, emphasizing a few points of special interest. The great variety of technical methods employed have brought to light many new features in the spermatogenesis of these Hemiptera, a few of which may be mentioned, but in the main they must be left for treatment in a later paper.

The general topography of spermiogenesis has been worked out on *Murgantia,* chiefly because the small size of the cells offers many advantages in studying the later stages, where at best the sperm heads are inconveniently long. The transformation of the spermatid nucleus into the sperm head has been described for *Euschistus* by Montgomery ('11) who carried his study to a stage which he called a mature sperm, describing the head "as a thin hollow cylinder of chromatin, containing a distinct cavity filled with nuclear sap." From his figures and his statement that "no evidence was found for the casting off of any substance by the sperm," it is abundantly clear that Montgomery failed to observe the final steps in the differentiation of the sperm head. In a stage following shortly on the last one described by Montgomery, the head undergoes a characteristic change resulting in what appears to be a complete vacuolization of the chromatic lining. Then the chromatin collapses toward the axis of the head, and a thin, compact thread is formed running throughout the length of the head and enclosed in a protoplasmic envelope which appears to correspond to the outline of the old head (Fig. 33). So far as I know, this stage in the transformation of the Hemipteran sperm has not been noted heretofore, but the description given by Faust ('13) of the mature sperm in *Anasa tristis* corresponds very closely to the stage just described in *Murgantia*. Possibly this phenomenon may prove to be much more generally distributed among the Hemiptera than has been suspected. That this is not a mature sperm is quite clear in my material, for subsequently the outer protoplasmic envelope disappears and the head of the completed spermatozoön is merely a rod of chromatin (Fig. 34) corresponding to the descriptions of many authors.

Such in brief is the course of events in the small cell generations of spermatids, but in the large cells it is quite different. In these, the layer of chromatin lining the nuclear membrane, so characteristic of the small spermatid, is from the first more or less vacuolated, and soon after the head begins to elongate (see Montgomery ('11), Fig. 141) the layer breaks down completely. The head now rapidly elongates, greatly exceeding the small sperm in this particular, while the chromatin content remains in an indefinitely vacuolated condition. Finally, the chromatin condenses into a more or less completely spiral thread which ultimately forms an axial rod as in the case of the small sperm, but of very much greater length (Fig. 35).

In brief, the two sizes of spermatids in *Murgantia* yield sperms of very different lengths by processes of nuclear differentiation which, while superficially unlike, prove to be fundamentally similar.

The centrioles in the Hemipteran spermatid have been so variously described, that a comparative analysis is out of the question in this paper. However, in view of the fact that I have been able to clear up some of the doubtful points in Montgomery's ('11) description of *Euschistus,* a very brief statement of the facts may be made as I have found them in *Murgantia.* In the very young spermatids the centriole is not single as Montgomery stated, but distinctly double (Fig. 22). This is especially clear at the time when the centrioles are migrating from their original anterior position to their definitive position between the nebenkern and the nuclear membrane. At this time they are always placed in a line normal to the nuclear wall, so that the proximal centriole touches the nucleus while the distal one gives rise to the axial filament of the tail (Fig. 22). Arrived in their position at the future base of the head, they undergo a change in orientation and for a short time their duplicity is lost, and only a single granule-like centriole can be distinguished. A little later, when the halves of the nebenkern have started to elongate, the two centrioles can again be made out in favorable cases. They appear in the form of two short rods, joined at one end to form a "V," at the vertex of which the axial filament is inserted (Fig. 26). At first the rods lie tangentially (or nearly so) to the nuclear membrane, and as seen from the side give the impression of a large granule to one edge of which the axial filament is attached, an asymmetry noted by Montgomery. Then the stray chromatin masses which have not taken part in the formation of the chromatin layer on the inner surface of the nuclear membrane begin to collect in the vicinity of the centrioles where they eventually form a compact and conspicuously staining body (Fig. 29). This body I have called the *pseudo-blepharoplast* because it has always been regarded as the centriole of the spermatozoön. Indeed the pseudo-blepharoplast so easily obscures the true centrioles, that lacking a study of earlier stages its real nature would certainly be overlooked. The rod-like centrioles eventually straighten out parallel to the long axis of the sperm (Fig. 29), and somewhat later the pseudo-blepharoplast fades away, as though it were dissolved in the nuclear sap. This phenomenon was correctly described by Montgomery who was naturally puzzled by the apparent solution of the spermatid centriole in the nuclear sap. The apparent anomalies of the Hemipteran centrioles are thus cleared up, and they are seen to conform with the usual types of sperm formation.

To summarize: (1) in the spermatid the customary pair of centrioles is present, which (2) remain in the "neck" region and give rise to the axial filament of the tail; (3) the centrioles in early stages are accompanied by a chromatic pseudo-blepharoplast, which is subsequently resorbed by the nuclear sap.

The fate of the spermatid mitochondria in *Euschistus* has been outlined by Montgomery ('11) and the much more complete account which I have worked out is not essential for the present purpose. Suffice it to say that the threads allotted to the spermatids by the second maturation division are rapidly condensed by characteristic steps into a compact sphere (Fig. 22) (the spermatid *nebenkern* of authors), which soon divides into two equal parts, and these, rapidly elongating, come to form a sort of mitochondrial sheath for the axial filament which lies between them. During the intermediate stages in the elongation of the nebenkern halves they develop a series of paired bleb-like swellings (Fig. 27) which increase in number as the mitochondrial sheaths spin out (Fig. 32) and are the most conspicuous thing in Benda preparations of this period. Subsequently they seem to be reabsorbed and the sheaths become mere threads running very nearly the entire length of the tail of the sperm. These vesicular swellings on the sheaths are to be found in a number of insects, as I have ascertained, but I find scarcely any direct mention of them in acounts of spermatogenesis; except in Duesberg's ('10) work on *Blatta* where something of a similar nature occurs. In the mature sperm the two sheaths appear to retain their thread-like structure showing no tendency to fuse into a complete mantle for the axial filament as is the case in *Blatta* according to Duesberg. Smears of mature sperm show clearly that the tail is flat and ribbon-like, each edge being formed of a distinct thread—the derivative in all probability of one of the original halves of the spermatid nebenkern.

I have, further, made some interesting observations on the structure of the mitochondria as revealed especially in the divided halves of the nebenkern during the early stages of their elongation. Students of the mitochondria in Mollusca and Lepidoptera have long supposed the chondriosomes to be composed of two different substances—an outer chromophilic envelope staining intensely with crystal violet and related stains, and an inner, medullary or "chromophobe" material which stains little or not at all with all the usual reagents. By means of Cajal's Golgi method I have succeeded (Bowen, '19) in impregnating this medullary substance with the greatest clearness, the chromophilic envelope being left quite colorless and transparent. Fuller treatment of these observations lies beyond the scope of this paper, but I would like to emphasize that a duplex chemical structure has thus been conclusively demonstrated at least for some mitochondria.

Finally, it remains to trace the rôle of the Golgi apparatus in the differentiation of the sperm—a subject to which a rather more complete treatment may be accorded by reason of its great theoretical importance. The preceding section gave an account of the Golgi apparatus up to the final anaphase of the second maturation division, when the daughter chromosome plates have completed their journey to the spindle poles and the dictyosomes are grouped in two clusters close to the chromosome plates and encircling the spindle remains (Fig. 18). I have followed out the further stages in both *Brochymena* and *Euschistus,* where they are essentially the same. In *Brochymena* the mitochondria seem to condense directly into the nebenkern, while the dictyosomes, already fusing with each other to form larger masses or Golgi bodies, are scattered over its whole available surface (Fig. 19). They are always thus closely in contact with the nebenkern, showing no tendency toward a more general distribution, and until the final fusion of all the Golgi elements they retain this close connection with the surface of the nebenkern. In *Euschistus,* on the contrary, the Golgi bodies while remaining in the vicinity of the nebenkern tend to be rather scattered.

The fusion of the Golgi bodies continues rapidly, their number constantly decreasing while their size correspondingly increases (Fig. 20). Soon definite little aggregates more or less plate-like or spherical in shape are formed whose periphery impregnates heavily just as did the Golgi bodies in the growth period, this heavily impregnated layer being characteristically absent on the side in contact with the nebenkern. In a short time the fusion has progressed so far that only three or four separate elements can be distinguished, and these too, continuing the process of fusion, soon unite to form a single mass of rather regular shape and dimensions (Fig. 21), which constitutes the "sphere" or idiosome of many writers on spermatogenesis. In the final stages of fusion the Golgi elements have tended to collect in the groove between the nuclear membrane and the nebenkern (Fig. 20), so that when its formation is completed, the single, condensed mass is always similarly situated (Fig. 21).

The dictyosomes distributed to the spermatids during the maturation divisions thus fuse to form a real Golgi apparatus, from which the acrosome is to be derived; and I therefore propose to call it the *acroblast*.[1] This term was first suggested by King ('07) and was subsequently adopted by Gatenby ('17) for designating in cells of various stages certain bodies supposed to give rise to the acrosome. The nature of these bodies in Miss King's work is doubtful, but in the case of Gatenby the acroblasts are certainly the Golgi bodies. It seems to me a procedure of little practical use and of very doubtful theoretical value to extend the term "acroblast" to structures present in the cell prior to the actual formation of the spermatid. In the first place, as I shall try to bring out later, it is highly probable that the Golgi apparatus is always the source of the acrosome, and it is clearly not desirable to use "acroblast" merely as a synonym for Golgi body or Golgi apparatus. Furthermore, cytologists are in actual want for some purely descriptive term which can be universally applied to the anlage of the acrosome without necessarily involving any implications as to origin and homology such as have so beclouded the terms "idiosome" and "sphere." I therefore propose to restrict the term *acroblast* to that body or bodies[2] from which the acrosome is actually derived.

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[1] My attention was first called to this very useful term by Professor Wilson.

[2] I use the word "bodies" because there are probably cases in which the acrosome is originally multiple, the parts later fusing into a single body, as for example in Lepidoptera, according to Gatenby ('17). In fact this occasionally happens in the Hemiptera, indicating that the small aggregates which finally fuse to form the definitive acroblast are each potentially acroblasts in miniature.

The acroblast is a more or less hemispherical body, pressed close to the nuclear membrane (Fig. 21), the periphery of which impregnates very heavily except for the portion touching the nucleus, where the substance which takes the osmic acid seems to be absent. The substance within the peripheral layer blackens very little and the picture as a whole recalls the similar arrangement of substances in the Golgi bodies of the growth period. The acroblast is completed at about the time the centrioles migrate around the nucleus to their position near the nebenkern, and is always located on the side of the nebenkern opposite that on which the centrioles will come to lie. As the centrioles migrate into their definitive position, the acroblast moves around the opposite side of the nucleus to an anterior position (Fig. 23). After pausing for a time (Fig. 24) it continues on around the nucleus, retracing the path over which the centrioles had traveled, and eventually comes to rest close to the centrioles (Fig. 25) but, with reference to its former position, on the opposite side of the nebenkern. Here it remains until the final step in the formation of the acrosome is completed.

As the acroblast slowly makes its way around the nucleus of the spermatid it undergoes a process of differentiation which, there is reason to believe, is of fundamental importance. The first evidence of this is the appearance at the base of the acroblast—that is at the surface next the nuclear membrane—of a transparent, spherical vesicle or bubble (sometimes there is more than one) which seems to be forming out of the colorless substance inside the acroblast (Fig. 23). At first it is very small but, growing rapidly, it gradually protrudes farther beyond the border of the acroblast which is thus lifted away from the nuclear membrane (Fig. 24). This transparent, structureless vesicle is to be the acrosome, and by this name it may be conveniently referred to even at this early stage in its formation. It is always in contact with the nuclear membrane while the acroblast fits over it like a shell. As the acrosome continues to enlarge, the acroblast seems to diminish somewhat in size, apparently at the expense of the non-staining substance, and eventually comes to form a mere appendage to the acrosome itself (Fig. 28). At the point where the acrosome touches the nucleus, a small granule is characteristically developed within the clear vesicle (Figs. 25 and 27), but this is not visible as a rule in Golgi preparations.

The spermatid is now ready to proceed with the general processes of differentiation. The acrosome shrinks somewhat, appearing to express some of its contents into the acroblast, and begins to move around the nucleus toward the anterior end (Fig. 30). As it does so its whole appearance changes; it becomes more opaque and begins to stain darkly with Fe-hematoxylin. Simultaneously the acroblast begins to draw away toward the tail region, and soon the connection with the remaining portion is severed (Fig. 31). The latter continues its journey around the nucleus, and becomes the acrosome while the former is carried back along the tail. The term *acroblast* is scarcely applicable to this cast off remnant (Fig. 31), nor is it any longer to be identified with certainty as Golgi apparatus, and I shall therefore refer to it merely as the "*Golgi remnant.*"[1]

For a while the Golgi remnant can be followed as it moves back along the tail, but it soon becomes lost in a group of granules from which it can not be certainly distinguished, and which are doubtless eventually cast out of the sperm with the protoplasmic ball sloughed off from the tail. It is practically certain that the Golgi remnant is cast off along with the other débris—at least I have never been able to find any traces of it in the last stage. Montgomery's ('11) statement that no protoplasm is eliminated from the sperm is certainly mistaken, for I have followed every step in the casting off of the protoplasmic balls and have ascertained that they are eventually ingested by the epithelial (nurse) cells which line the cavity of each cyst.

CRITICAL CONSIDERATIONS.

I. *Problems of Cytoplasmic Division.*

As yet our knowledge of exact processes in the distribution of the formed elements of the cytoplasm in cell division is exceedingly limited and we are, indeed, accustomed to call attention to the contrast between the accurate, meristic division of the nucleus and the supposedly haphazard, mass division of the cytoplasm as one of the striking features of karyokinesis. In part, no doubt, we have been led to an easy acceptance of this view by what appear to be the necessary implications of the modern chromosomal theory of heredity, which, by its emphasis on the exactness of chromosome distribution, perhaps predisposes us to assign a secondary importance to the division of the specific components of the cytoplasm. Such a standpoint is unwarranted, for while it may be improbable that any mechanism will ever be made out for an exact division of the cytoplasm comparable to that of the nucleus, it does not necessarily follow that cytoplasmic division can be reduced to the basis of a mere mass division subject only to the laws of chance. Nevertheless, at the present time cytoplasmic division seems often to be treated as if it were a haphazard process, the essential phenomena of which are the same whether cell division be mitotic or amitotic, with of course an implied exception in the case of the centrioles and the achromatic division figure.

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[1] This cast off body is homologous with the *idiophthartosome* of Papanicolaou and Stockard ('18) and the *idiozomrest,* etc., of many authors. We are really much in need of a convenient and purely descriptive term for this body.

It seems to me, however, that the facts observed in the Hemiptera point rather to a conception of cytoplasmic division as a precisely ordered process. That the cytoplasm may indeed undergo division of a remarkably regular character has long been recognized in the special case of the segmenting egg; and in the so-called "determinate" eggs particularly of molluscs and ascidians the experimental studies of recent years have revealed a very definite order in the distribution of cytoplasmic factors of differentiation which presumably are traceable to corresponding "formative stuffs." For accomplishing such complicated cytoplasmic divisions some special mechanism seems necessary, but as to its nature very little has been made out. In the division of tissue cells, however, appeal can not be made, as in the case of the egg, to direct experiment, and we must here rely on morphological studies alone. An approach to the problem may therefore be made by a comparison of the quantitative relations existing between the products. Accordingly the problem of cytoplasmic division may be stated for present purposes in the form of a double question: (1) Is the division of the cytoplasm and its formed elements strictly regular as regards the quantities involved? (2) If so, what is the mechanism for achieving such a result?

For an answer to these questions we can have recourse to no more suitable material than is offered by the two spermatocyte divisions. In these cases, if anywhere, we should expect to find conclusive evidence of an exact regularity in division, for the spermatocyte divisions and the subsequent differentiation of the sperm are accomplished so rapidly that there is little time for the correction of inequalities in division by differential growth.

As a matter of fact the mass distribution of the cytoplasmic elements has been studied by many workers with very uniform results. Attention has thus far been centered chiefly on the mitochondria, and Duesberg ('10) has well summarized this aspect of the question, showing conclusively that the spermatids arising from a given spermatocyte contain each a fourth part of its original mitochondrial content. This general conclusion is corroborated with the greatest clearness by the case of the scorpion *Centrurus* described by Wilson ('16) in which each spermatid receives accurately equal portions of a remarkable chondriosome-ring characteristic of the primary spermatocytes. To be sure, in another scorpion, *Opisthacanthus,* the distribution is sometimes demonstrably uneven, but the discrepancies are always slight. My own observations on the Hemiptera show that the mitochondria are rather accurately halved at each maturation division, with the result that each spermatid receives an approximately equal mass of chondriosome material. On the other hand there are cases in which the distribution is markedly unequal, especially in forms in which the spermatocyte divisions are abnormal, as for example the honey-bee (Meves, '07). Of even greater interest are the inequalities in the mass distribution of the mitochondria in the segmenting eggs of the ascidian (Duesberg, '15). It is clear, therefore, that the division of the mitochondria considered merely from the standpoint of the quantities involved is very often equal, but in certain special cases quite the contrary. In any particular case, however, the distribution appears to be always approximately the same. In other words, there is a definite *regularity* in the amount of mitochondria distributed to a given cell.

In the case of the Golgi apparatus, the evidence is as yet very meager, but none the less positive. Gatenby ('18) has recently given some account of dictyokinesis in several genera of pulmonates, and in the *Limacidæ* at least he found evidence of a very exact distribution of the Golgi elements, corroborating the similar observations of Platner ('89) made many years ago. Although his observations were not made on the germ cells, Deineka ('12) has given one of the clearest accounts of dictyokinesis so far published, and he points out the apparently equal distribution of the dictyosomes to the daughter cells. The observations on Hemiptera which I have already given furnish, it seems to me, the best ground on which to base an opinion; for the essentials of dictyokinesis are so clear that it is possible to arrive at a reliable conclusion. And indeed it may be affirmed that the Golgi apparatus of the spermatocytes is divided very equally among the spermatids, so that each receives a fourth of the original amount. Accordingly, in the case of the Golgi apparatus there is also a definite regularity in the quantities distributed in division to the daughter cells.

Concerning the mass of cytoplasm proper, an approximately equal division often occurs, at other times a very unequal one, but in any case the same regularity is a characteristic feature. Summing up the evidence, therefore, our first question concerning the existence of regularity in the mass division of the cytoplasm may be answered affirmatively. There *is* conclusive evidence that the cytoplasm and its principal formed elements are distributed with great regularity by the processes of cell division, the most usual result being that each daughter cell receives a rather accurate half of the total quantity of cytoplasmic materials.

Concerning the second question, namely, as to the mechanism by which this regularity in mass distribution is achieved, two answers at once suggest themselves: (1) The division may be a purely chance separation of elements distributed at random in the cytoplasm; or (2) It may be accomplished by definite activities related to those which produce the spindle and the resultant phenomena of karyokinesis. Thus far the relation of the cytoplasmic elements to the centrioles and the spindle in general has received scant attention, and the idea of chance distribution has been rather tacitly accepted as an explanation. My observations on *Euschistus* do not bear out this view, and I should like therefore to make a brief survey of the problem as a whole in an effort to find, if possible, a consistent basis for the whole series of observations.

It has often been pointed out that the chondriosome-material in dividing cells (spermatocytes, and others) tends to appear in the form either of granules or of rods and threads of various dimensions. In fact one can arrange these various morphological types in a rather instructive series. There is first the case in which the chondriosomes are scattered rather evenly throughout the cytoplasm in the form of small granules, as, for example, in the guinea pig (Duesberg, '10). An orderly arrangement with respect to the achromatic division figure here seems entirely lacking. In many pulmonates (Gatenby, '18) conditions are very similar except that the granules are much larger, and in many Lepidoptera (Gatenby, '17) enlarge to form vesicular bodies, in some cases few in number and of considerable size. From such a condition it is a short step to that in the scorpion *Opisthacanthus,* where a definite(?) number of "chondriosome spheres" occur, involving a further element of regularity which seems lacking in the other cases mentioned. All the chondriosome formations so far considered agree in one particular; they do not appear to be subject to any influence other than that of chance. Only in a single case, so far as I know, has a definite orientation of small scattered chondriosomes been described. This is the condition in *Ascaris* described by Hirschler ('13) where the slightly elongate chondriosomes are all distinctly oriented toward the centrioles. This observation should be confirmed, for if correct it throws much light on the whole mechanism by which granular chondriosomes are distributed in cell division.

The chondriosome-ring of *Centrurus* offers an interesting introduction to the rod type of mitochondria, for after becoming drawn out along the spindle it breaks apart into two rod-like halves which are subsequently divided across and the parts distributed to the daughter cells. It is only a step from this case to that of *Paludina* (Gatenby, '19*a*) where there is a limited number of heavy rods which become arranged along the spindle in the telophase and as in the ring derivatives of *Centrurus* are subsequently divided. In these two cases there appears for the first time a relationship between the spindle and the chondriosomes which is essential to an equal, mass division. In *Blaps* (Duesberg, '10) the mitochondrial rods are very numerous and much smaller in diameter, and, what is of most interest, are oriented toward one pole of the cell during the prophases of the first maturation division. What relation this bears to the centrioles is not stated, but the arrangement is clearly a forerunner of the "palisade" of rods which encloses the spindle during division, and is divided across as in the two preceding cases. A further step in the definite orientation of the mitochondria with respect to the spindle is found in the case of the honey-bee described by Meves ('07). In the bee the early history of the mitochondria is very similar to that which I have described in *Euschistus,* the late growth period being characterized by a tangled mass of threads enveloping the nucleus on all sides. During the prophases, however, the threads are all aggregated into a single tangled mass situated opposite that centriole which ultimately comes to lie in the functional sperm, and therefore presumably in the vicinity of the other centriole. As the spindle forms, free ends of threads begin to appear around the edge of the mass of mitochondria; and these gradually move toward the opposite centriole until the whole mass is untangled and the threads stretch from centriole to centriole, parallel to the spindle and enclosing it more or less completely in a mantle of threads. Wilke ('13) has also described the orientation of mitochondria toward the centrioles in the spermatocyte divisions of *Hydrometra,* but his figures give a rather inadequate idea of the exact chain of events. Finally, the case of *Euschistus,* described in an earlier section, demonstrates conclusively that the arrangement of the mitochondria preparatory to division depends in these forms upon some influence that is as it were focused in the centrioles. There is thus a complete series ranging from a mass of scattered granules dependent for equal division apparently on the exigencies of chance, up to a mass of threads definitely oriented towards the centrioles to the end that an equal division of their substance may occur. How can these apparent contradictions be harmonized? A possible answer to this question may be postponed until the mechanism of dictyokinesis has also been considered.

Although the Golgi apparatus has been known for over twenty years there is still scarcely a single adequate account of its exact behavior in cell division. Duesberg's ('20) recent report of the Golgi apparatus in the opossum and the account by Papanicolaou and Stockard ('18) of the guinea pig, leave much to be desired in the way of details, and it is difficult to draw any very trustworthy conclusions. It appears, however, that in these forms the Golgi apparatus undergoes a simple fragmentation, the resultant pieces being scattered throughout the cell and subsequently divided in a way reminiscent of the chondriosome granules, for example in the guinea pig. A similar distribution of previously separate Golgi bodies has been described in *Ascaris* by Hirschler ('13). Practically all other accounts agree in indicating a much more complicated process. Platner ('89) seems to have been the first to study the division of the Golgi apparatus in detail. He worked on the pulmonate *Limax*—material which has since been extensively employed for the same purpose since its Golgi apparatus seems especially resistant to acetic acid. Platner observed the breaking up of the "Nebenkern" just prior to division and the separation of the pieces into two groups which, accompanying the centrioles, migrate to opposite sides of the nucleus and when the spindle is formed become disposed more or less radially around the centriole. At the second spermatocyte division, the same process is repeated. Platner also described some interesting division phenomena of the individual rods (dictyosomes?), which have not however been confirmed in detail by later workers. The essential features to be noted in this description are the fragmentation of the parts of the Golgi apparatus and their distribution to the daughter cells seemingly under the direct control of the centrioles. Since Platner's time many others have examined the so-called "Nebenkern" of pulmonates with most contradictory results, and it must be confessed that none succeeded as well as did Platner in gaining a complete conception of dictyokinesis. Of later workers, Murray ('98) should be especially mentioned, for his observations on *Helix* and *Arion* contain many details relative to the structure of the dictyosomes which offer most interesting comparisons with my own observations on the Hemiptera. He failed to confirm Platner's description of a very accurate splitting of each dictyosome during the division stages, but his work bears out the conclusion that some sort of fragmentation occurs like that so clearly demonstrated in *Euschistus*. Fragmentation of the original Golgi bodies or "batonettes" is also indicated by the difficulty which Gatenby ('18) experienced in staining the Golgi elements during the division stages. I have had the same experience with *Euschistus,* in which the Golgi bodies stain readily with Fe-hematoxylin after the proper technique but the dictyosomes fail entirely of demonstration. Gatenby ('17) also noted the aggregation of his "acroblasts" (probably dictyosomes) around the centrioles during the spermatocyte divisions of many Lepidoptera but how they attain this position was not indicated.

Deineka ('12) has described the division of the Golgi apparatus in cells of Descemet's membrane, and the general outline is distinctly similar to the type of behavior in *Euschistus*. The dictyosomes formed by fragmentation of the Golgi reticulum are arranged in an equatorial belt encircling the metaphase chromosome-plate, and thence they wander to the opposite poles of the spindle, becoming in this way divided into two distinct polar groups. Finally, in *Euschistus,* I have been able to follow the process step by step, and the relation of dictyokinesis to the general mitotic figure is completely demonstrated.

If we try now to gain some general conception of the processes by which cytoplasmic division is accomplished, we note in the first place one fundamental fact applicable to all cases. *The cytoplasmic elements are so distributed during the early division stages that the constriction of the cell wall will ultimately divide them in some regular mass proportion.* It has been held that this distribution is a matter of chance only, but I believe that the facts set forth above point rather to the existence of a definite mechanism for the regular arrangement of the cytoplasmic elements in division. Indeed, the case of *Euschistus* provides convincing evidence that the mitochondria and Golgi apparatus may undergo a division accomplished in some way by activities that focus in the centrioles. The evidence from other sources tends to the same conclusion in respect to the division of the Golgi apparatus, except possibly in mammals where the descriptions are contradictory. With the mitochondria however the case is different, and there seems on the surface to be no general rule. May this not be, after all, only an apparent difference? It seems difficult to conceive of a purely chance distribution of mitochondria which would produce the relatively constant results in *Opisthacanthus* for example, and it is still more difficult to conceive of a chance arrangement which would allow the dividing cell to sever the chondriosome-ring of *Centrurus* into exactly equal parts. Why should chance distribute the granules in the guinea pig germ cell so evenly? Why, indeed, should the granules be distributed at all, since in earlier stages they tend to be aggregated at one pole of the cell? In this connection the observation of Meves ('14) on the dividing egg of *Ascaris* is of interest, for he found that the mitochondria in the form of very small granules tended to collect around the poles of the cleavage spindles, *i.e.*, around the centrioles. Finally there is the case of the spermatocyte divisions of *Ascaris* in which the axial dimensions of the mitochondria reveal the presence of an underlying polarization which, if the chondriosomes were spherical, would never be suspected.

If we combine these many facts, it is difficult to escape the conviction that the division of the mitochondria and the Golgi apparatus is always in more or less definite relation to the centrioles and that the regularity which has been shown to exist in cytoplasmic distribution is thus to be traced ultimately to the same influences which control the formation of the mitotic figure and the distribution of the chromosomes. I have come thus to a concept of the dividing cell as a whole which differs somewhat from that now commonly accepted. It seems to me probable that the centriole is to be considered not merely the morphological expression of the influences which construct the spindle and marshal the chromosomes for division, but that it is in a much wider sense the dynamic center of the whole cell, in which are centered at the time of division influences that extend to practically all the elements of the cell and direct their division in an orderly manner. This view of the functional significance of the centriole takes us back again to the theories long ago proposed by Van Beneden and Boveri, which contained a nucleus of real truth that later workers have been inclined to overlook.

This conclusion leads naturally to the consideration of a further possibility, namely, that the division of the cytoplasmic structures may be actually more or less meristic in character. It is impossible in the present state of our knowledge to make any very definite statement on this point, which has thus far received little careful study. The results from the study of plant plastids indicate something, however, of the possibilities for growth, multiplication, and distribution in cell division possessed by these bodies. All that is needed to give such behavior the aspect of a meristic division is a definite mechanism for the distribution of the plastids in mitosis. That such an apparatus may exist is demonstrated clearly by the surprisingly complicated mode of division undergone by the Golgi bodies in *Euschistus,* in which a partially meristic division almost certainly occurs. Such facts as these coupled with the probable relation of many cytoplasmic elements to a mechanism for orderly division indicate a possible degree of cytoplasmic organization which has been little suspected. Unfortunately our knowledge concerning it is as yet so meager that we can scarcely even conjecture its nature and extent, but of its possibilities for a highly complex mode of division there can, I believe, be no longer any doubt.

II. *The Origin of the Acrosome.*

No structure in the spermatid has been subject to more contradictions of description and homology than the acrosome.[1] It has in general been traced to a "sphere," the relations of which to preceding cell structures have generally been merely inferred and often quite mistaken. But aside from the purely descriptive "sphere," the acrosome has been asserted to originate from the mitochondria, spindle remnants, and combinations of mitochondria and spindle remnants, from a centrosome, a centriole, an accessory chromosome, an acroblast, new formations in the cytoplasm, an idiosome of doubtful ancestry, and the Golgi apparatus. In the vertebrates especially, where there is a very definite “sphere” or idiosome in the spermatocytes, the similar formation in the spermatid has been referred to the spermatocyte structures —generally without any adequate reasons. It is among the insects, where a spermatocyte “idiosome” seems rarely to be well developed, that the greatest confusion has prevailed. Indeed any attempt to extract some fundamental conception from the maze of description has thus far seemed hopeless. Viewing the problem as a whole, two facts alone stand out in seemingly universal agreement, namely, (1) that there is always a body which comes to lie at the anterior tip of the sperm and forms the acrosome of authors, and (2) that in connection with this process there is usually a remnant cast off and lost in the protoplasm of the tail region.

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[1] The term "Akrosoma" was first applied by Lenhossék ('98) to the little granule within the vesicle which is produced by the "sphere" (acroblast). As a matter of fact the term has been applied so widely to the material as a whole which forms the apical body that I see little reason for attempting to restrict the word to Lenhossék's original meaning.

The second of these may require some notes in explanation. From a general survey of spermatogenesis, I have found fairly clear evidence of the elimination of some material formerly in connection with the developing acrosome in many cases, of which the following are a representative selection from widely separated groups:

Nematoda		*Ascaris* (Hirschler, ’13)
Insecta	Coleoptera	*Passalus* (Schafer, ’17)
		Cybister (Voinov, ’03)
	Orthoptera	*Gryllotalpa* (Payne, ’16)
		Locusta (Otte, ’07)
	Hemiptera	*Pyrrhocoris* (Henking, ’91)
		Anasa (Paulmier, ’99)
		Euschistus (Montgomery, ’11)
Arachnida		*Lycosa* (Boesenberg, ’05)
Mollusca		*Columbella* (Schitz, ’16)
		Paludina (Gatenby, ’19*a*)
Amphibia		*Bufo* (King, ’07)
Mammalia		*Mus* (Niessing, ’97, v. Lenhossék, ’98)
		Cavia Benda, ’91, Niessing, ’97, v. Lenhossék, ’98, Sjoevall, ’06, and others
		Didelphys (Duesberg, ’20)

Other cases can readily be found in which the casting off of a remnant by the acrosome has not been mentioned but is nevertheless indicated (see for example Terni, '14). If then the anlage of the acrosome presents this bipartite character, how is the acrosome itself derived and what is the nature of the cast off remnant? I believe that the facts which I have described in the Hemiptera offer a decisive answer to this question, and at the same time point the way to a new conception of the acrosome which may clear up the confusion in which we find ourselves at present.

In *Euschistus* we find in the primary spermatocytes a number of scattered Golgi bodies characterized by a duplex structure. On the one hand there is a heavily stained peripheral zone, the "Golgi apparatus" of authors, and on the other a central non-staining or chromophobe substance to the nature of which I will return later. In the maturation divisions these bodies undergo fragmentation into dictyosomes which, having been distributed in equal amounts to the spermatids, undergo progressive fusion until a single mass (acroblast) is formed, the "sphere" of many authors. This mass presents the same staining affinities as did the Golgi bodies of the spermatocytes, a darkly staining peripheral layer and a chromophobe interior. Although it has not been possible to offer conclusive proof of the *exact* homology of these two substances in spermatocyte and spermatid, I think it can scarcely be doubted that we have in this mass (acroblast) the direct reconstitution of the structure of the spermatocyte Golgi bodies. In other words, *the material (acroblast) from which the acrosome is to be derived is the Golgi apparatus.*[1] By a process of differentiation connected with the chromophobe material occupying the interior of the acroblast (*Golgi apparatus*), and probably at its expense, there is formed a large, vesicular body containing a small granule, which together form the acrosome of the sperm. The remnant of the Golgi apparatus is cast off and appears to take no further part in the formation of the sperm, while the acrosome is applied to the anterior part of the sperm head and may there undergo further changes the nature of which is of no interest in this connection.

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[1] I have used the term *Golgi apparatus* as covering the Golgi bodies (and acroblast) as a structural whole, a usage which seems to be justified for the present in the light of the possibilities which are discussed in the following section on the structure of the Golgi complex. As I have there pointed out, the restriction of the term *Golgi apparatus* to the part impregnated by the customary methods is very possibly an artificial limitation.

To draw a general conclusion from the facts in the pentatomids alone might well seem an unwarranted procedure; but fortunately more or less fragmentary accounts from several other animal groups are now available, and they corroborate fully the general outline which I have given. The best case is that of the opossum where Duesberg ('20) has described the origin of a vesicular acrosome with an enclosed granule from the Golgi apparatus which he proved to be directly derived from the "idiosome" of the spermatocytes. Once the acrosome is formed, the Golgi remnant is cast off. A similar though less complete account has been given by Sjoevall ('06) for the guinea pig. From Terni's ('14) figures of *Geotriton* it seems certain that much the same thing occurs in that amphibian. Further, the work of Schitz ('16) and Gatenby ('19*a*) clearly proves the same thing in several molluscs, though in this case the acrosome is in the form of a granule the exact homologies of which are as yet uncertain. Gatenby ('17) has also described the origin of the acrosome from Golgi bodies (his acroblasts) in the Lepidoptera, though the case is in some respects contradictory and not yet entirely clear. Lastly, Hirschler ('13) has described the partial elimination of the Golgi apparatus from the sperm of *Ascaris,* the remainder apparently forming a body which some writers have compared with the acrosome of typical nematosperms. The failure of many workers to trace the origin of the acroblast is obviously due to the difficulty of demonstrating the Golgi apparatus during cell division, and the fantastic descriptions especially among the insects are clearly the result of inadequate fixing and staining methods. From a study of a wide variety of technical methods I can testify to the great differences which are attributable to methods of preparation alone.

Drawing together these many threads of evidence, both from observation and inference, it seems to me that the following conclusion embodies a conception of the acrosome which is in accord with all the facts and which establishes its formation as an orderly part of a logical scheme of sperm formation. The acrosome of the animal sperm arises in connection with the Golgi apparatus. When the differentiation of the acrosome has proceeded to a certain point, the remnant of the Golgi apparatus is cast off and probably plays no essential rôle in the mature sperm. The acrosome is, therefore, no fortuitous body contrived at the last minute from spindle fibers or other chance inclusions, but is to be considered in the light of a cell organ as definite in its origin and rôle as are the nucleus, centrioles and mitochondria of the spermatid.

III. *The Idiosome and the Structure of the Golgi Apparatus.*

In conclusion I would like to refer briefly to some general problems connected primarily with the structural aspects of the Golgi apparatus in the male germ cells. In the first place it is to be noted that the structures especially characteristic of the spermatocytes of pulmonates and vertebrates and appearing in the literature under the name of Golgi apparatus, idiosome, Nebenkern, sphere and other less familiar terms, are all one and the same thing in a topographical sense. They represent a complex of the Golgi apparatus plus a definite substance which forms the idiosome of Meves. Duesberg ('20) has pointed out, and with reason, that the idiosome has nothing to do with the old attraction sphere of Van Beneden, or, let me add, with the archoplasm which is supposed to form the spindle and astral rays of the dividing cell. The idiosome of Meves was supposed to be a mass of protoplasm surrounding the centrioles in the resting cell. In other words, it derived its essential character from its relation to the centrioles. Where then, it is fair to ask, is the idiosome in the insect spermatocyte? Obviously there is none. Fortunately the spermatids furnish a clue which leads to a logical solution of the whole problem. In the vertebrate and pulmonate spermatid there is seldom, if ever, any relation between the idiosome and the centrioles, but there is a constant relation between the idiosome and the Golgi apparatus, a relation which applies as well to the condition seen in the insects. In the spermatocytes of insects, however, the material of the spermatid "idiosome" is scattered throughout the cytoplasm as a part and parcel of each

Golgi body. In other words the densely staining part of each Golgi body is equivalent to the "Golgi apparatus" of authors, while the non-staining substance associated with it is equivalent to the "idiosome" of authors. If the Golgi bodies of the insect could be aggregated into a single mass such as that in the pulmonate "Nebenkern," then we would have the typical idiosome and the typical Golgi apparatus of so many workers. The insects thus furnish a clue to the whole involved series. The non-staining substance which forms the idiosome is really related not to the centrioles, which may be situated within it at certain times, but to the Golgi apparatus. So far as we know at present this intimate relation seems never to be broken. The idea that the "idiosome" is somehow related to the centrioles seems to have been fostered by the view that "idiosome" and "archoplasm" are related, and that the idiosome could thus share in the formation of the spindle. This is certainly negatived in the Hemiptera where the Golgi bodies have nothing to do with the spindle, and by the further fact that in at least some mammals the idiosome is still intact in the metaphase of division when the spindle is fully formed.

I would suggest therefore that before we theorize further on these most confusing structures, a thorough reexamination of the whole subject be made, using as a working hypothesis the possibility that the Golgi apparatus and the idiosome may be essentially a single structural complex. We know that in some cases at least the mitochondria are composed of two distinct substances, one staining readily by the usual methods, the other little if at all. So too the Golgi complex would seem to be similarly duplex in its structure, composed on the one hand of a readily staining substance (the Golgi apparatus) and on the other of a relatively non-staining material (the idiosomic substance). Should further research bear out this possibility, the confusion in which we have been involved will be dissipated and we shall have laid a firm foundation on which to base the future study of these cytoplasmic structures of the germ cells.

SUMMARY.

1. In *Euschistus* the mitochondrial material of the primary spermatocytes occurs in the form of threads which are definitely oriented toward the centrioles in the prophases of the maturation divisions.

2. Due to this very definite arrangement, the threads are so distributed during the anaphases that each daughter cell contains one half of the mitochondrial material.

3. The mitochondria are not divided autonomously on the spindle but as a result of their position or the mechanical action of the constricting cell wall.

4. The Golgi apparatus of the spermatocytes is extensively developed, occurring in the form of scattered Golgi bodies.

5. During the maturation divisions these bodies undergo a process of autonomous fragmentation into dictyosomes which are arranged in a definite relation to the spindle and are then distributed in equal groups to the daughter cells. The arrangement and distribution of the Golgi elements during division is clearly dependent upon the centrioles.

6. In the spermatids the Golgi elements are condensed into a single body, the acroblast, from which the acrosome of the mature sperm is derived.

7. The remnant of the acroblast (or Golgi remnant) ultimately breaks free from the acrosome and is lost in the cytoplasm of the tail probably taking no further part in the formation of the sperm.

8. The centrioles of the spermatid are, as in most (all?) animals, paired, and in this case remain in the "neck" region of the mature sperm.

On the basis of the facts brought out in this study it is suggested that:

1. The regularity in the division of the cytoplasmic elements of the cell, as illustrated by the mitochondria and Golgi bodies of *Euschistus,* is not the result of chance but is accomplished by a definite mechanism focused in the centrioles, and may perhaps partake of the nature of a meristic, as contrasted to a mass division.

2. The acrosome of the animal sperm is probably universally

formed in connection with the Golgi apparatus, and has nothing whatever to do with spindle fibers or other cell parts. It is to be considered as much a characteristic part of the sperm, having a definite morphological value, as are the nucleus and mitochondria.

3. The idiosome plus the Golgi apparatus of vertebrates and pulmonates is to be considered as the homologue of the scattered Golgi bodies of insects, the idiosomic substance and the Golgi material being in some way intimately related, possibly in the sense of an essentially single cell organ of duplex chemical nature.

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EXPLANATION OF PLATES.

All of the figures have been outlined as far as possible with the camera lucida at an initial enlargement of approximately 3800 diameters. At so great an enlargement it has of course been necessary to correct the outlines extensively and to add much of the finer detail free hand. In reproducing, the figures have been reduced uniformly to an enlargement of approximately 2300 diameters. In every case the method employed in the preparation of the original object has been indicated.

A, acrosome
a, acroblast
c, centrioles
ch, chromosomes
G, Golgi remnant
N, nebenkern
n, nucleus
p, pseudo-blepharoplast

Plate I.

Fig. 15 is from *Brochymena quadripustulata;* Figs. 4 and 6 are from *Euschistus variolarius;* the others are from *Euschistus euschistoides.* All the figures are from cells of the small (" normal ") generations except 3, 4, 5, 6 and 7.

Fig. 1. Late spermatogonium (or stage *a* of Wilson ('12)). The Golgi bodies are aggregated around the faintly stained " cap " of mitochondria. (Modified Kopsch.)

Fig. 2. Late diplotene or early growth period. Fusion of Golgi bodies prior to their migration throughout the cytoplasm. (Modified Kopsch.)

Fig. 3. Diplotene. Mitochondrial " cap " resolved into threads. (Benda.)

Fig. 4. Very early growth period. Initial steps in the distribution of the Golgi bodies. (Modified Kopsch.)

Fig. 5. Early growth period. Distribution of the mitochondria completed. (Benda.)

Fig. 6. Early growth period. Distribution of the Golgi bodies completed. (Modified Kopsch.)

Fig. 7. Late growth period. Growth of Golgi bodies almost completed. (Modified Kopsch.)

Fig. 8. Middle prophase. Slightly oblique polar view of girdle of Golgi bodies. Composite from two adjacent sections of the same cell. (Modified Kopsch.)

Fig. 9. Late prophase. Lateral view of a part of the ring of dictyosomes encircling the nucleus. (Modified Kopsch.)

Fig. 10. Late prophase. Polar view of the ring of dictyosomes encircling the nucleus. (Modified Kopsch.)

Fig. 11. Late prophase. Lateral view. (Benda.)

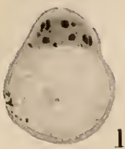
Fig. 12. Late prophase. Polar view. (Benda.)

Fig. 13. Late prophase. Oblique section through one polar field. (Benda.)

Fig. 14. First maturation division metaphase. (Benda.)

Fig. 15. First maturation division metaphase. Dictyokinesis completed. (Modified Kopsch.)

Fig. 16. First maturation division anaphase. Composite from two adjacent sections of the same cell. (Benda.)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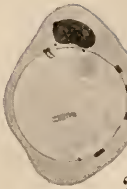
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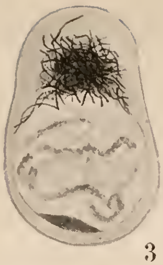
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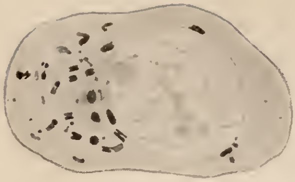
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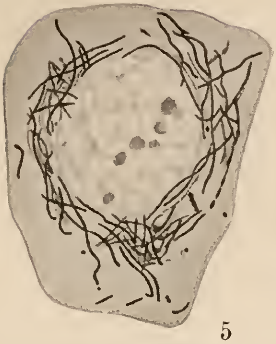
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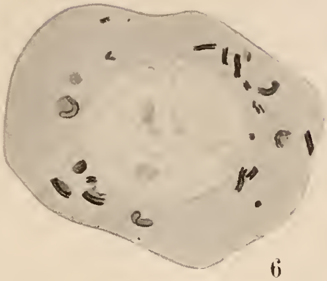
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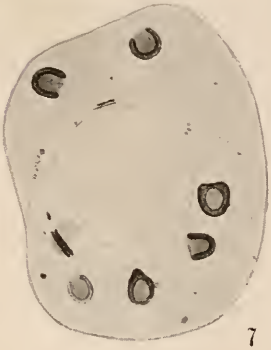
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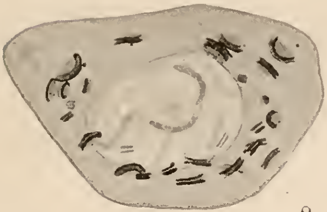
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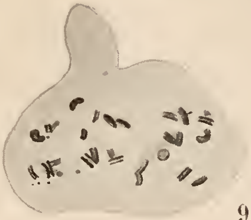
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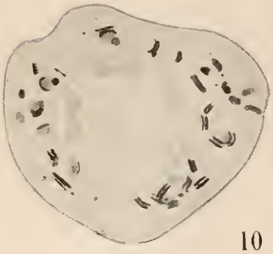
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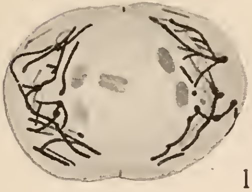
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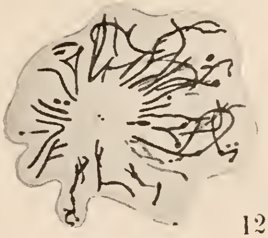
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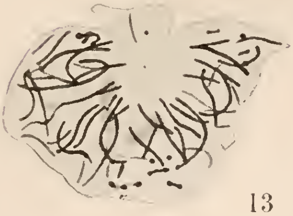
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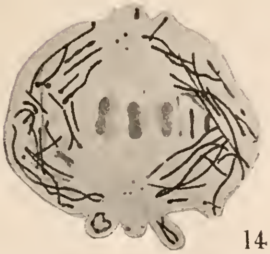
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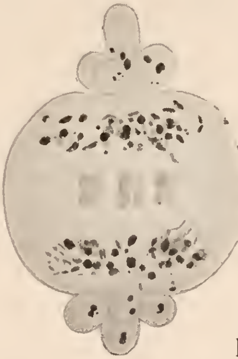
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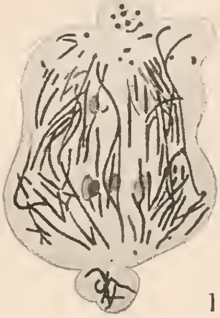
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R. H. BOWEN.

Plate II.

Fig. 30 is from *Euschistus servus;* Figs. 17, 23 and 24 are from *Euschistus euschistoides;* Figs. 18 to 21, 28 and 31 are from *Brochymena quadripustulata;* the others are from *Murgantia histrionica.* All the figures are from cells of the small ("normal") generations except 23, 24, 30 and 35.

Fig. 17. First maturation division telophase. Division of the mitochondrial "palisade" practically complete. (Benda.)

Fig. 18. Second maturation division telophase. (Modified Kopsch.)

Figs. 19, 20, 21. Spermatids. Progressive stages in the aggregation of the Golgi elements to form the acroblast. (Modified Kopsch.)

Fig. 22. Spermatid. Centrioles double, and the tail filament in first stage of its growth. (Benda.)

Fig. 23. Spermatid. First appearance of the vesicular acrosome within the acroblast. (Modified Kopsch.)

Fig. 24. Spermatid. Later stage in the differentiation of the acrosome. (Modified Kopsch.)

Fig. 25. Spermatid. Nebenkern halves elongating; typical granule developed in the acrosome. (Benda.)

Fig. 26. Spermatid. Centrioles in "V" formation. (Flemming-hematoxylin.)

Fig. 27. Spermatid. First steps in formation of swellings on the mitochondrial sheaths. (Benda.)

Fig. 28. Spermatid. Late stage in differentiation of the acrosome. (Modified Kopsch.)

Fig. 29. Spermatid. Pseudo-blepharoplast completely formed; centrioles straightened out along the main axis of the sperm. (Flemming-hematoxylin.)

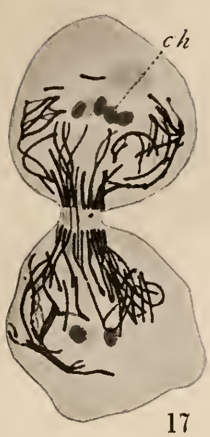
Figs. 30, 31. Spermatids. Final steps in separation of the acrosome from the acroblast (Golgi remnant). (Modified Kopsch.)

Fig. 32. Spermatid. Head and portion of the tail with the characteristic swellings on the mitochondrial sheaths. (Benda.)

Fig. 33. Immature sperm head. The chromatin forms a thin axial rod enclosed in a protoplasmic envelop. (Flemming-hematoxylin.)

Fig. 34. Mature sperm head from small cell generation. (Flemming-hematoxylin.)

Fig. 35. Mature sperm head from large cell generation. (Hermann-hematoxylin.)



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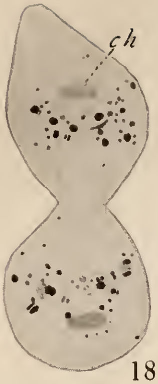

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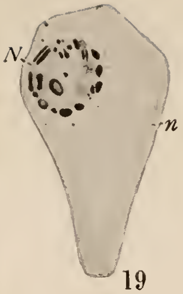

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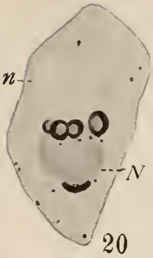

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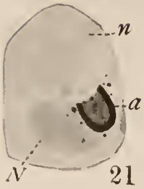

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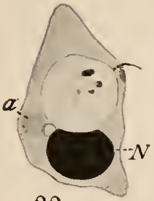

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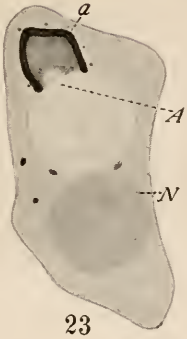

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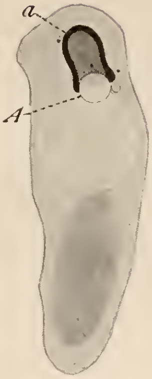

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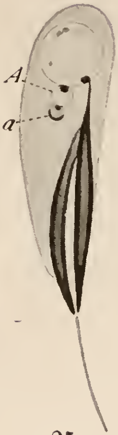

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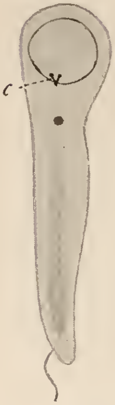

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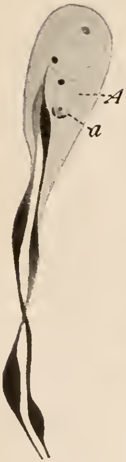

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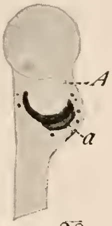

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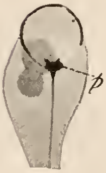

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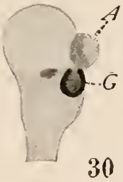

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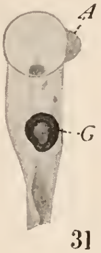

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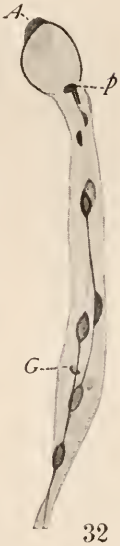

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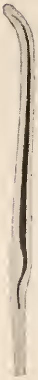

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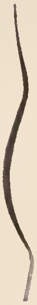

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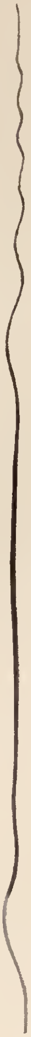

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